Kurc et al.: Invasive pulmonary aspergillosis diagnosis in hematology patients

Invasive pulmonary aspergillosis evaluation in hematology patients: Three years results of tertiary hospital

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ABSTRACT

Invasive pulmonary aspergillosis (IPA) is the most frequent invasive fungal disease occurring in patients with hematological malignancies. Serum galactomannan (GM) antigen monitoring is thought to be helpful in the diagnosis of IPA. The aim of this study was to determine the role of a GM assay in serum samples for the diagnosis of IPA in patients with hematological disease. The data of 366 immunosuppressed patients that were hospitalized and followed up in the hematology clinic from January 2017 to December 2019 were retrospectively analyzed. The clinical and radiological findings of the patients and the GM results, requested twice a week, were evaluated. In this study, the incidence of probable and possible IPA was determined to be 15.3% (56/366). Of the cases detected, 28 (50.0%) were patients diagnosed with Acute Myeloid Leukemia (AML), and 34 (60.7%) patients who had compatible clinical and examination findings were started on antifungal treatment. Additionally, AUC (Area Under the Curve) values were calculated by ROC (Receiver Operating Characteristic) analysis, and it was determined that the diagnostic efficiency was more predictive when the cut-off was 0.5 in the GM test for IPA disease. The detection of GM antigen in serum is a very useful and rapid method for diagnosing IPA disease in immunosuppressed hematology patients. However, GM results should be evaluated together with clinical and radiological findings for early diagnosis, and the treatment approach should be determined accordingly.

Keywords: Invasive pulmonary aspergillosis, galactomannan testing, hematological malignancy
INTRODUCTION

Aspergillus is defined as a fungus belonging to the Ascomycota phylum with multicellular, branched structures called hyphae. Aspergillus species are organisms that are capable of proliferating in nitrogen and carbon sources, are characterized as thermotolerant (proliferates at 37-50°C) due to their ribosomal proteins and are resistant to high osmotic pressures [1]. As a commonly occurring filamentous fungus in the environment and in areas where health services are provided, Aspergillus spreads to susceptible persons through its aerial conidia. Although conidium present in the environment is inhaled daily, majority of people do not develop diseases related to Aspergillus. This is because the normal human immune system has developed its defense through alveolar macrophages, neutrophils, monocytes and natural killer cells [2].

Aspergillus species are encountered as an agent in many clinical pictures. This leads to invasive aspergillosis and superficial infections (otomycosis, keratitis and burn injury infections), which affects more than 300000 individuals annually, in addition to allergic bronchopulmonary disease and rhinosinusitis affecting more than 10 million individuals globally and chronic pulmonary and rhinosinusital aspergillosis affecting approximately 3 million individuals [3].

Rapid diagnosis is crucial in Invasive Pulmonary Aspergillosis (IPA) due to high mortality and morbidity rates, especially in severely immunosuppressed patients with limited host defence including solid organ transplantation, hematological malignancies and neutropenia [2,4]. However, difficulties in diagnosis are present due to non-specific clinical-radiological findings, invasive sampling requirements, and low sensitivity of traditional culturing and histopathological methods [5]. Galactomannan Antigen (GM) test presents a potential non-invasive diagnostic method. However, some antifungals and antibiotics can affect the precision of the GM test, occasionally giving false positive or false negative results [4].

According to the guidelines of Infectious Diseases Society of America, serum and Bronchoalveolar Lavage (BAL) GM detection was reported to provide high quality evidence in diagnosing invasive aspergillosis in adult or pediatric patients with hematological malignancies or undergoing hematopoietic stem cell transplantation and is strongly suggested as the correct marker. While routine serum GM screening is not advised in patients taking antifungal treatment or prophylaxis, GM test is advised in bronchoscopic materials [6].
However, combined application of weekly GM test and computerized tomography (CT) are suggested to be effective in patients receiving antifungal treatment [4]. Whereas, GM is not advised for screening in solid organ recipients and those suffering from chronic granulomatous disease. Moreover, 1,3-Beta-D-Glucan test is advised in invasive aspergillosis diagnosis, but it is not a specific test for diagnosing Aspergillus [6]. GM sensitivity is considerably lower in non-neutropenic patients when compared to neutropenic patients. GM Optical Density Index (ODI) levels are a reliable indicator for determining the success of antifungal treatments [7].

This study aimed to investigate and compare the diagnostic value of serum GM antigen test in immunosuppressed patients hospitalized in our hematology clinic and suspected of having IPA, in addition to determining the optimal GM cutoff value in our patient group.

MATERIALS AND METHODS

Patient Group

The data belonging to hospitalized patients who were treated and followed-up in the hematology clinic of Tekirdağ Namık Kemal University Hospital between the years 2017-2019 were retrospectively analyzed in our study. The patients' clinical and radiological findings and the GM results requested twice weekly were evaluated.

Demographic data, radiological and clinical findings, extended antibiotic use, risk factors such as neutropenia, histopathology, and fungal biomarkers of the patients were analyzed. Additionally, the number of total GM serum tests and the number of positive and negative results of the patients were analyzed. Antifungal treatments given for IPA despite the negative test results were also recorded.

Radiological findings such as pulmonary infiltrations, consolidation, halo sign, cavity or air-crescent sign, cavitation, lesions with well-defined boundaries, and dense nodules were evaluated in terms of invasive aspergillosis in the high resolution lung CT scans of all patients. According to these data, the occurrence of clinical findings in patients with suspicious lung lesions were accepted.

GM testing

GM test was carried out on serum samples collected from patients at least twice a week using Sandwich-ELISA method and Platelia Aspergillus Ag kit (Platelia™ Asper-gillus, Bio-Rad,
USA) according to the manufacturer’s instructions. The ODI value of the samples were measured by the microplate reader, and then GM test results in the serum samples were calculated. GM test results with ODI value of 0.5 and above were regarded as positive.

**IPA diagnostic criteria**

The European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) 2008 criteria were used to diagnose patients with IPA [8]. Patients were categorized as having no IPA, proven, probable or possible IPA.

According to these criteria: (i) Proven IPA; presence of hyphae in histopathological and direct microscopic examinations, presence of Aspergillus sp. growth in the culture; (ii) probable IPA; The presence of, at least one of the host factors (neutropenia, solid organ transplant, connective tissue disorders, or usage of immunosuppressive agents, such as corticosteroids), at least one of the clinical criteria (a halo sign, an air-crescent sign or cavity) and mycological criteria (such as a positive Aspergillus sp. culture from qualified specimens or a positive serum GM detection result at a cutoff value of ≥ 0.5), (iii) Possible IPA; The presence of at least one host factor and one clinical criterion, but absence of mycological criteria.

**Ethical statement**

This study was approved by the Non-Interventional Clinical Research Ethics Committee of Tekirdağ Namık Kemal University, the approval number 2019/111/07/07 (27.06.2019). We confirm that the study was conducted in accordance with the relevant guidelines/regulations.

**Statistical analysis**

The results obtained in the study were statistically analyzed using SPSS 20.0 (SPSS Inc., Chicago, IL, USA) package program. Descriptive statistics are presented as mean±standard deviation for numeric variables and as number (n) and percentage (%) for categoric variables. Non-parametric Spearman's correlation test was applied to evaluate the relationship between data. Kruskall-Wallis test was used for detecting the difference between quantitative variables of patients diagnosed and not diagnosed with IPA regarding the GM test, and Mann-Whitney U test was used to detect the differences between the two groups. Study data were evaluated at 95% confidence interval and two-way. A value of p<0.05 was accepted for statistical significance. Finally, a Receiver Operating Characteristic (ROC) curve was constructed to determine the optimum cut-off value for the patient group's GM test.
RESULTS

Patient characteristics

Patients receiving treatment and follow-up at our hospital's Hematology Clinic between the years 2017-2019 constitute our study group. A total of 366 patients with ages varying between 16-90 (58.57±16.85), 174 (47.5%) of which are female and 192 (52.5%) of which are male, were included in our study. In 79 (21.6%) of the cases AML (Acute Myeloid Leukemia), in 59 (16.1%) MM (Multiple Myeloma), in 51 (13.9%) NHL (Non-Hodgkin Lymphoma (aggressive lymphoma (60.8% of 51 patients) and indolent lymphoma (39.2% of 51 patients)), and in 32 (8.7%) CLL (Chronic Lymphocytic Leukemia) constituted the most common underlying diseases, while in 67 (18.3%) of the cases some other hematological diseases (haemophagocytic syndrome, undiagnosed cause unknown leukocytosis and leukopenia , idiopathic thrombocytopenic purpura, polycythemia vera, myelodysplastic syndrome) and in 33 (9.0%) of the cases some anaemias (iron deficiency anemia, anemia, aplastic anemia, hemolytic anemia, megaloblastic anemia) constituted the underlying diseases (Table 1). An abnormal finding was identified in high-resolution CT findings in 94 (25.7%) of the cases. Of the 128 patients with GM positive results, 57 (44.5%) were present in this group.

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GM testing results and antifungal treatment

A total of 2053 GM tests were conducted at different times on the patient group (n= 366). GM positive results were detected in 128 (35.1%) of the patients. Recurrent positive results were identified in 45 (35.1%) of these 128 patients. There was radiological evidence in 57 (44.5%) of the GM-positive patients, while 79 (61.7%) had neutropenia.

In our study, when the GM test results of our patient group were evaluated, the most common underlying disease was AML (n=79). Of these patients, 40 had a positive GM test and 33 had neutropenia. Additionally, it was observed that the GM test was negative in 39 patients and neutropenia was found in 25 patients.

Antifungal treatment was initiated in 53 (41.4%) of patients with positive serum GM tests. 38 (29.7%) of these received posaconazole, 7 (5.4%) received voriconazole, 6 received
(4.7%) fluconazole, and 2 (1.6%) received anidulafungin treatment, while 3 of them received antifungal agents due to different reasons. Moreover, antifungal treatment was initiated by clinicians on 23 patients although their GM antigen test result was negative (n=238) (Table 2).

In our study, patients whose GM test results were positive and negative were compared from various aspects. Accordingly, a significant relationship was found between positivity in the GM test and both initiation of antifungal treatment and the presence of abnormal findings on CT scans (p < 0.001).

**Patients diagnosed with IPA**

Since biopsy or needle aspiration biopsy samples were not obtained for histopathological and culture examination in any of the patients, a definitive diagnosis of IPA could not be established according to EORTC/MSG criteria.

The number of patients diagnosed with probable IPA was detected as 44 (12.0%) and those with possible IPA was 12 (3.3%). The 56 (15.3%) patients who received probable or possible IPA diagnosis had ages ranging between 19-88 (57.03±15.4), of these 39 were male (69.6%) and 28 (50.0%) were patients diagnosed with AML. Among them, 34 (60.7%) patients with consistent clinical and examination findings were diagnosed with IPA and antifungal treatment was initiated. However, antifungal treatment was started in 76 (20.8%) of the patients with and without a diagnosis of IPA. In our study, 310 (84.7%) patients did not receive a diagnosis of IPA according to the EORTC/MSG criteria (Figure 1, Table 2).

In this study, GM positive results were detected in 128 of all patients. Of these patients; while 44 were diagnosed with IPA, 84 were not diagnosed with IPA. When the GM ODI value was evaluated, it was seen that the proportion of patients with an ODI value ≥ 0.5 was high in both groups (Table 3). In addition, recurrent GM test positivity was detected in 45 of the patients diagnosed with IPA, and in 24 patients in the other group.

In our study, the mortality rate in patients diagnosed with IPA according to EORTC/MSG criteria was determined to be 25% (n = 14) and 6.12% (n = 19) in patients not diagnosed with IPA.

**Detection of Optimal cut-off value effect for GM testing**
The ROC curve according to the different GM cut-off values (ODI value: 0.5, 1.0, 1.5, 2.0) was constructed in our study, AUC (Area Under the Curve) values were calculated for diagnostic efficiency in IPA disease, and these values were determined to be more decisive in terms of diagnostic efficiency at a cut-off of 0.5 (AUC: 0.757; standard error, 0.035; 95% confidence interval, 0.689 to 0.826). However, it was found that sensitivity and specificity were highest at the accepted value, and as the cut-off value increased, a decrease in sensitivity was determined (Figure 2).

**DISCUSSION**

IPA is considered a disease of immunocompromised patients [2]. However, clinical and radiological findings are also not specific. The challenges in diagnosis arise from the requirement of invasive procedures for a definitive diagnosis and the frequent presence of thrombocytopenia and coagulation disorders in patients. Fungal culture on the other hand is time consuming and has low positivity rates. Therefore, non-invasive and rapid diagnostic methods are used [4,5,9]. GM, Beta-D glucan, PCR, Aspergillus lateral flow, urinary antigen, siderophores, cytokines and pentraxin-3 detection, PET/CT and immuno PET/MRI are among these methods [10-17]. The use of different diagnostic methods simultaneously increases the accuracy of the diagnosis [10,18]. Among these, GM is a cell wall polysaccharide of *Aspergillus* species and, although it proliferates in invasive infections, it can be detected in serum and other body fluids. It can be detected in the circulation 5-8 days before clinical symptoms appear. Additionally, two consecutive test results with an ODI value $\geq 0.5$ are required for the highest test accuracy [19]. Moreover, 2 serum GM tests must be carried out weekly to initiate preemptive treatment that increases survival [18]. IPA is seen more frequently in AML patients when compared to other patient groups [4, 18, 20]. However, risk factors such as diabetes mellitus, severe influenza disease, and chronic granulomatous disease are also reported [18]. Invasive diagnostic methods are not preferred for patient monitoring in our hospital's hematology clinic due to the risk of complications. Among non-invasive tests, GM is used twice weekly. GM tests were conducted on 366 patients hospitalized in our clinic with various hematological diagnoses, and AML patients accounted for 21.6% of them. Positive results were detected in 128 (35.1%) of the patients and 45 (35.1%) of these had recurrent positive results. Among patients diagnosed with IPA and initiated on antifungal treatment (n=26), recurrent positivity was detected in 80.8% (n=21) of cases.
While the diagnosis of IPA is increasingly recognized, many cases go unnoticed, and diagnosis can only be made post-mortem [18,21]. The EB-A2 monoclonal antibody is used to detect the β-1,5 galactopyranosyl antigenic side chain in the GM ELISA test [22]. In high-risk patients, false negativity in the GM test can occur due to the use of mold active antifungal prophylaxis [10,20]. Additionally, sensitivity is low in non-neutropenic patients [7,19]. False positives may be seen due to reasons such as beta lactam antibiotic use (piperacillin-tazobactam, amoxicillin-clavulanate), transfusion, plasmalyte infusion, Histoplasmososis, Fusariosis, *Bifidobacterium* spp., Multiple myeloma, severe mucositis, IV immunoglobulin, *Aspergillus* contaminated foods [10,19,22]. The rate of false positive results was determined as 14% in a study where the importance of considering fungi other than *Aspergillus* as well was emphasized [23]. Similarly, false positive results were detected in 21 (5.7%) patients not diagnosed with IPA in our study. Despite numerous factors that may influence the results, strong recommendation and high-quality evidence suggest the use of BAL and serum GM measurements [6].

Host factors and clinical criteria compliant with EORTC/MSG criteria are used besides GM testing [8]. Twice-weekly GM screening in neutropenic patients, combined with the use of clinical and radiological findings, is currently considered the most appropriate approach for diagnosis [6]. The sensitivity of serial GM screening is quite high in neutropenic patients [7]. Weekly GM measurements and CT monitoring are effective in the early diagnosis of IPA even in febrile neutropenic patients receiving antifungal treatment [4]. In our study, 44 (12.0%) patients with GM positive results, neutrophil count <500/mm³, and positive CT findings were diagnosed with probable IPA. While 12 (3.3%) patients with GM negative results, neutrophil count <500/mm³, and positive CT findings were diagnosed with IPA. The percentage of patients diagnosed with IPA according to different studies was reported as 6.1-13.4% [24, 25, 26, 27]. A total of 15.3% of the patients in our study undergoing follow-up for GM were diagnosed with IPA. According to our study results, it is observed that GM positivity is consistent with radiological findings in patients considered to have IPA. In a study, when the presence of radiological findings was compared with GM positivity, it was determined that GM positivity was highly consistent with radiological findings [28]. These data suggest that GM positivity before the detection of radiological findings may be helpful in diagnosing IPA.

Although studies on *Aspergillus* species indicate that the rate of resistance to some antifungals (azole resistance) varies [29, 30, 31, 32], in another study report no resistance to
voriconazole and amphotericin B [31]. Resistance rates differ according to the *Aspergillus* species. In one study, Amphotericin B resistance rates were reported as 11.8% in for *A. fumigatus*, 10% in *A. flavus*, and 33.3% in *A. niger*; Itraconazole resistance was 11.8% in *A. fumigatus*, 20% in *A. flavus*, and 33.3% in *A. niger*, while no resistance to caspofungin was observed. The mortality rate in aspergillosis has been reported as 26.7% [33]. According to another study, the mortality rate was reported as 29.2% in the group treated with voriconazole and 42.1% in the group treated with amphotericin B [34]. In our study, among the 56 patients diagnosed with probable or possible IPA according to the EORTC/MSG criteria, mortality rate was determined as 25% (n=14) in patients diagnosed with IPA based on clinical findings and initiated treatment.

It has been reported that the rate of decrease in GM in response to antifungal treatment is important in terms of mortality. [6,7,10]. Patients who successfully responded to voriconazole treatment had earlier decreases in GM values compared to those whose response failed at the end of treatment [6]. High mortality rate (55.5%) in patients receiving voriconazole (n=9) at our hospital is especially noteworthy. GM levels must be monitored in treatment follow-up as well in order to reduce the mortality rate and if the expected reduction is not observed, highly azole resistant non-fumigatus species must be considered as a probable causative agent of IPA.

Clinically significant ODI cut-off determination is essential in terms of clinical application for patients with hematologic malignancies due to their high mortality rates. One important factor influencing the test's sensitivity and specificity is the choice of cutoff point for positivity, which determines whether or not results qualify as true positives. In our study, the generated ROC curve confirmed that the test had a good performance, even at low cutoffs. Similar to previous studies, a high overall sensitivity and an acceptable specificity was obtained by using GM ODI value at a cutoff of 0.5 to define an IPA case.

The most significant limitation of our study is the lack of species identification and sensitivity testing in Aspergillosis due to the inability to perform invasive procedures. However, high mortality rates in patients receiving voriconazole treatment suggests that voriconazole resistance may be high and that non-fumigatus *Aspergillus* species may also be involved.
CONCLUSION

In conclusion, patients can be monitored using CT findings and non-invasive, rapid GM testing due to the difficulty in implementing invasive procedures required for a definitive diagnosis which is associated with the risk of complications, and the time-consuming nature of fungal culturing and its low positivity rate. Additionally, local epidemiological data should be continuously monitored to reduce mortality, and treatment approaches should be determined accordingly.

ACKNOWLEDGMENTS

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[33] Roohani AH, Fatima N, Shameem M, Khan HM, Khan PA, Akhtar A. Comparing The Profile of Respiratory Fungal Pathogens Amongst Immunocompetent and Immunocompromised Hosts, Their Susceptibility Pattern and Correlation of Various Opportunistic Respiratory Fungal Infections and Their Progression in Relation to the

TABLE 1. Demographic Characteristics of the Patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>192 (52.5%)</td>
</tr>
<tr>
<td>Women</td>
<td>174 (47.5%)</td>
</tr>
<tr>
<td>Age (mean), Years (range)</td>
<td>58.57±16.85 (16-90)</td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
</tr>
<tr>
<td>AML</td>
<td>79 (21.6%)</td>
</tr>
<tr>
<td>MM</td>
<td>59 (16.1%)</td>
</tr>
<tr>
<td>NHL</td>
<td>51 (13.9%)</td>
</tr>
<tr>
<td>CLL</td>
<td>32 (8.7%)</td>
</tr>
<tr>
<td>ALL</td>
<td>18 (4.9%)</td>
</tr>
<tr>
<td>HL</td>
<td>8 (2.2%)</td>
</tr>
<tr>
<td>CML</td>
<td>7 (1.9%)</td>
</tr>
<tr>
<td>HCL</td>
<td>6 (1.6%)</td>
</tr>
<tr>
<td>Solid organ malignancy</td>
<td>6 (1.6%)</td>
</tr>
<tr>
<td>Other hematological disease</td>
<td>67 (18.3%)</td>
</tr>
<tr>
<td>Anemia</td>
<td>33 (9.0%)</td>
</tr>
</tbody>
</table>

TABLE 2. IPA diagnoses, GM test results, CT, neutropenia and antifungal treatment findings of patients

<table>
<thead>
<tr>
<th>Classification of Fungal Infection</th>
<th>No of Patient</th>
<th>GM testing</th>
<th>CT Finding</th>
<th>Neutropenia</th>
<th>Antifungal treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>IPA cases</td>
<td>56 (15.3%)</td>
<td>44 (78.6%)</td>
<td>12 (21.4%)</td>
<td>56 (100%)</td>
<td>56 (100%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>34 (60.7%)</td>
</tr>
<tr>
<td>Non IPA cases</td>
<td>310 (84.7%)</td>
<td>84 (27.1%)</td>
<td>226 (72.9%)</td>
<td>38 (12.2%)</td>
<td>90 (29.0%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>42 (13.5%)</td>
</tr>
<tr>
<td>All Patients</td>
<td>366 (%100)</td>
<td>128 (35%)</td>
<td>238 (65%)</td>
<td>94 (25.7%)</td>
<td>146 (39.9%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>76 (20.8%)</td>
</tr>
</tbody>
</table>

TABLE 3. Distribution of GM ODI values of patients with and without a diagnosis of IPA

<table>
<thead>
<tr>
<th>Variable</th>
<th>IPA cases (n=56)</th>
<th>Non IPA cases (n=310)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM positive results</td>
<td>44 (100%)</td>
<td>84 (100%)</td>
</tr>
<tr>
<td>ODI ≥ 0.5</td>
<td>16 (36.4%)</td>
<td>45 (53.6%)</td>
</tr>
<tr>
<td>ODI ≥ 1.0</td>
<td>11 (25.0%)</td>
<td>14 (16.7%)</td>
</tr>
<tr>
<td>ODI ≥ 1.5</td>
<td>2 (4.5%)</td>
<td>6 (7.1%)</td>
</tr>
<tr>
<td>ODI ≥ 2.0</td>
<td>15 (34.1%)</td>
<td>19 (22.6%)</td>
</tr>
</tbody>
</table>
FIGURE 1. Scatter diagram displaying the GM ODI value of patients diagnosed with IPA

FIGURE 2. ROC curve for serum GM detection in patients with IPA