Biomolecules and Biomedicine
ISSN: 2831-0896 (Print) | ISSN: 2831-090X (Online)
Journal Impact Factor® (2023): 3.1
CiteScore® (2023): 7.4
www.biomolbiomed.com |blog.biomolbiomed.com

RESEARCH ARTICLE

TRANSLATIONAL AND CLINICAL RESEARCH

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

Mine Aydın Kurc[1]*, Betül Günaydın[2], Seval Akpınar[3], Birol Safak[4], Nuri Kiraz[5]

[1]Department of Medical Microbiology, Tekirdag Namik Kemal University, Tekirdag, Türkiye.

[2]Microbiology Laboratory, Uşak Training and Research Hospital, Usak, Türkiye.

[3]Department of Internal Diseases, Tekirdag Namik Kemal University, Tekirdag, Türkiye.

[4]Department of Medical Microbiology, Atlas University, Istanbul, Türkiye.

[5]Department of Medical Microbiology, Istanbul University, Istanbul, Türkiye.

***Corresponding author:** Mine Aydın Kurc; Email: mineaydines@gmail.com.

DOI: https://doi.org/10.17305/bb.2024.10766

Submitted: 22 May 2024/ **Accepted:** 14 July 2024/ **Published online:** 17 July 2024

Conflicts of interest: Authors declare no conflicts of interest.

Funding: Authors received no specific funding for this work.

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 radio-logical 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 pa-tients 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 hypae 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 be-tween 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.

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.

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 ≥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, sensitivty 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, plasmalyt infusion, Histoplasmosis, 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 $<500/mm^3$, and positive CT findings were diagnosed with probable IPA. While 12 (3.3%) patients with GM negative results, neutrophil count $<500/mm^3$, 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

The authors would like to thank Prof. Dr. Gamze Varol for statistical analysis.

REFERENCES

[1] Szalewski DA, Hinrichs VS, Zinniel DK, Barletta RG. The Pathogenicity of Aspergillus fumigatus, Drug Resistance, and Nanoparticle Delivery. Can J Microbiol. 2018;64(7): 439-453. doi: 10.1139/cjm-2017-0749.

[2] Shah MM, Hsiao EI, Kirsch CM, Gohil A, Narasimhan S, Stevens DA. Invasive Pulmonary Aspergillosis and Influenza Co-infection in Immunocompetent Hosts: Case Reports and Review of The Literature. Diagn Microbiol Infect Dis. 2018;91(2):147-152. doi: 10.1016/j.diagmicrobio.2018.01.014.

[3] Richardson M, Bowyer P, Sabino R. The Human Lung and Aspergillus: You Are What You Breathe in? Med Mycol. 2019(1); 57(Supplement2), S145-S154. doi: 10.1093/mmy/myy149.

[4] Wang X, Guo G, Cai R, He P, Zhang M. Utility of Serum Galactomannan Antigen Testing Combined with Chest Computed Tomography for Early Diagnosis of Invasive Pulmonary Aspergillosis in Patients with Hematological Malignancies with Febrile Neutropenia After Antifungal Drug Treatment. J Int Med Res. 2019;47(2):783-790. doi: 10.1177/0300060518811472.

[5] Singh S, Kaur H, Choudhary H, Sethi S, Malhotra P, Gupta KL, Rudramurthy SM, Chakrabarti A. Evaluation of Biomarkers: Galactomannan and 1,3-beta-D-glucan Assay for The Diagnosis of Invasive Fungal Infections in Immunocompromised Patients from A Tertiary Care Centre. Indian J Med Microbiol. 2018 Oct-Dec; 36(4):557-563. doi: 10.4103/ijmm.IJMM_18_366. PMID: 30880706.

[6] Patterson TF, Thompson GR, Denning DW, Fishman JA, Hadley S, Herbrecht R, Kontoyiannis DP, Marr KA, Morrison VA, Nguyen MH, Segal BH, Steinbach WJ, Stevens DA, Walsh TJ, Wingard JR, Young JA, Bennett JE. Practice Guidelines for the Diagnosis and Management of Aspergillosis: 2016 Update by the Infectious Diseases Society of America. Clin Infect Dis. 2016 Aug 15; 63(4): e1-e60. doi: 10.1093/cid/ciw326.

[7] Ullmann AJ, Aguado JM, Arikan-Akdagli S, Denning DW, Groll AH, Lagrou K, Lass-Flörl C, Lewis RE, Munoz P, Verweij PE, Warris A, Ader F, Akova M, Arendrup MC, Barnes RA, Beigelman-Aubry C, Blot S, Bouza E, Brüggemann RJM, Buchheidt D, Cadranel J, Castagnola E, Chakrabarti A, Cuenca-Estrella M, Dimopoulos G, Fortun J, Gangneux JP, Garbino J, Heinz WJ, Herbrecht R, Heussel CP, Kibbler CC, Klimko N, Kullberg BJ, Lange C, Lehrnbecher T, Löffler J, Lortholary O, Maertens J, Marchetti O,

Meis JF, Pagano L, Ribaud P, Richardson M, Roilides E, Ruhnke M, Sanguinetti M, Sheppard DC, Sinkó J, Skiada A, Vehreschild MJGT, Viscoli C, Cornely OA. Diagnosis and Management of Aspergillus Diseases: Executive Summary of the 2017 ESCMID-ECMM-ERS Guideline. Clin Microbiol Infect. 2018 May; 24 Suppl 1:e1-e38. doi: 10.1016/j.cmi.2018.01.002.

[8] De Pauw B, Walsh TJ, Donnelly JP, Stevens DA, Edwards JE, Calandra T, Pappas PG, Maertens J, Lortholary O, Kauffman CA, et al. Revised Definitions of Invasive Fungal Disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. Clin Infect Dis. 2008;46:1813–1821. doi: 10.1086/588660.

[9] Erdem İ, Doğan M, Karaali R, Elbasan Omar Ş, Ardiç E. Treatment of Invasive Aspergillosis. Namık Kemal Medical Journal. 2018; 6(2):64 – 82.

[10] Paiva JA, Pereira JM. Biomarkers of fungal lung infection. Curr Opin Infect Dis. 2019;32(2):136-142. doi: 10.1097/QCO.0000000000000523.

[11] Hoenigl M, Orasch T, Faserl K, Prattes J, Loeffler J, Springer J, Gsaller F, Reischies F, Duettmann W, Raggam RB, Lindner H, Haas H. Triacetylfusarinine C: A Urine Biomarker for Diagnosis of Invasive Aspergillosis. J Infect. 2019 Feb;78(2):150-157. doi: 10.1016/j.jinf.2018.09.006.

[12] Oesterreicher Z, Eberl S, Zeitlinger, M. Impact of Different Antimycotics on Cytokine Levels in An In vitro Aspergillosis Model in Human whole Blood. Infection. 2020 Feb;48(1):65-73. doi: 10.1007/s15010-019-01346-x.

[13] Marr KA, Datta K, Mehta S, Ostrander DB, Rock M, Francis J, Feldmesser M. Urine Antigen Detection as an Aid to Diagnose Invasive Aspergillosis. Clin Infect Dis. 2018 Nov 13; 67(11): 1705-1711. doi: 10.1093/cid/ciy326.

[14] Hoenigl M, Eigl S, Heldt S, Duettmann W, Thornton C, Prattes J. Clinical Evaluation of The Newly Formatted Lateral-flow Device for Invasive Pulmonary Aspergillosis. Mycoses. 2018 Jan; 61(1): 40-43. doi: 10.1111/myc.12704.

[15] Li H, Liu L, Zhou W, Rui Y, He B, Shi Y, Su X. Pentraxin 3 in Bronchoalveolar Lavage Fluid and Plasma in Non-neutropenic Patients with Pulmonary Aspergillosis. Clin Microbiol Infect. 2019 Apr;25(4):504-510. doi: 10.1016/j.cmi.2018.06.015.

[16] Cruciani M, Mengoli C, Barnes R, Donnelly JP, Loeffler J, Jones BL, Klingspor L, Maertens J, Morton CO, White LP. Polymerase Chain Reaction Blood Tests for The

Diagnosis of Invasive Aspergillosis in Immunocompromised People. Cochrane Database Syst Rev. 2019 Sep 3; 9:CD009551. doi: 10.1002/14651858.CD009551.pub3.
[17] Jenks JD, Salzer HJF, Hoenigl M. Improving The Rates of Aspergillus Detection: An Update on Current Diagnostic Strategies. Expert Rev Anti Infect Ther. 2019;17(1):39-50. doi: 10.1080/14787210.2018.1558054.
[18] Danion F, Rouzaud C, Duréault A, Poirée S, Bougnoux ME, Alanio A, Lanternier F, Lortholary O. Why Are So Many Cases of Invasive Aspergillosis Missed? Med Mycol. 2019 Apr 1; 57(Supplement2):S94-S103. doi: 10.1093/mmy/myy081.
[19] Lass-Flörli C. How to Make A Fast Diagnosis in Invasive Aspergillus. Med Mycol. 2019; 1, 57(Supplement2): S155-S160. doi: 10.1093/mmy/myy103.
[20] Magira EE, Jiang Y, Kontoyiannis DP. Baseline Serum Aspergillus Galactomannan Index in Patients with Hematologic Malignancy and Culture-documented Invasive Pulmonary Aspergillosis: Is there A Difference Among Aspergillus species? Med Mycol. 2019; 57:639–642. doi: 10.1093/mmy/myy109.
[21] Adad Tejerina EE, Abril E, Padilla R, Rodríguez Ruíz C, Ballen A, Frutos-Vivar F, Lorente JÁ, Esteban A. Invasive Aspergillosis in Critically Ill Patients: An Autopsy Study. Mycoses. 2019; 62(8):673-679. doi: 10.1111/myc.12927.
[22] Patterson TF, Donnelly JP. New Concepts in Diagnostics for Invasive Mycoses: Non-Culture-Based Methodologies. J Fungi (Basel). 2019(17); 5(1). doi: 10.3390/jof5010009.
[23] Maertens J, Verhaegen J, Lagrou K, Van Eldere J, Boogaerts M. Screening for Circulating Galactomannan As A Noninvasive Diagnostic Tool for Invasive Aspergillosis in Prolonged Neutropenic Patients and Stem Cell Transplantation Recipients: A prospective validation. Blood 2001;97(6):1604-10. doi: 10.1182/blood.v97.6.1604.
[24] Tavakoli M, Yazdani Charati J, Hedayati MT, Moosazadeh M, Badiee P, Seyedmousavi S, Denning DW. National Trends in Incidence, Prevalence and Disability-Adjusted Life Years of Invasive Aspergillosis in Iran: A Systematic Review and Meta-analysis. *Expert Rev Respir Med*. 2019 Nov; 13(11):1121-1134. doi: 10.1080/17476348.2019.1657835.
[25] Blot SI, Taccone FS, Van den Abeele AM, Bulpa P, Meersseman W, Brusselaers N, Dimopoulos G, Paiva JA, Misset B, Rello J, Vandewoude K, Vogelaers D, AspICU Study Investigators. A Clinical Algorithm to Diagnose Invasive Pulmonary Aspergillosis in Critically Ill Patients. Am J Respir Crit Care Med. 2012;186(1):56-64. doi: 10.1164/rccm.201111-1978OC.

[26] Linke C, Ehlert K, Ahlmann M, Fröhlich B, Mohring D, Burkhardt B, Rössig C, Groll AH. Epidemiology, Utilisation of Healthcare Resources and Outcome of Invasive Fungal Diseases Following Paediatric Allogeneic Haematopoietic Stem Cell Transplantation. Mycoses 2019;29. doi: 10.1111/myc.13029.

[27] Linke C, Tragiannidis A, Ahlmann M, Fröhlich B, Wältermann M, Burkhardt B, Rossig C, Groll AH. Epidemiology and Management Burden of Invasive Fungal Infections After Autologous Hematopoietic Stem Cell Transplantation: 10-year Experience at A European Pediatric Cancer Center. Mycoses 2019 Oct;62(10):954-960. doi: 10.1111/myc.12968.

[28] Pazos C, Pontón J, Del Palacio A. Contribution of (1-3)-beta-D-glucan Chromogenic Assay to Diagnosis and Therapeutic Monitoring of Invasive Aspergillosis in Neutropenic Adult Patients: A Comparison with Serial Screening for Circulating Galactomannan. J Clin Microbiol. 2005;43(1): 299-305. doi: 10.1128/JCM.43.1.299-305.2005.

[29] Van der Linden JW, Snelders E, Kampinga GA, Rijnders BJ, Mattsson E, Debets-Ossenkopp YJ, Kuijper EJ, Van Tiel FH, Melchers WJ, Verweij PE. Clinical Implications of Azole Resistance in Aspergillus fumigatus, The Netherlands, 2007-2009. Emerg Infect Dis. 2011;17(10):1846-54. doi: 10.3201/eid1710.110226.

[30] Chen YC, Kuo SF, Wang HC, Wu CJ, Lin YS, Li WS, Lee CH. Azole Resistance in Aspergillus Species in Southern Taiwan: An epidemiological surveillance study. Mycoses. 2019;62(12):1174-1181. doi: 10.1111/myc.13008.

[31] Monteiro C, Pinheiro D, Maia M, Faria MA, Lameiras C, Pinto E. Aspergillus Species Collected from Environmental Air Samples in Portugal-Molecular Identification, Antifungal Susceptibility and Sequencing of cyp51A Gene on A. fumigatus sensu stricto Itraconazole Resistant. J Appl Microbiol. 2019 Apr;126(4):1140-1148. doi: 10.1111/jam.14217.

[32] Özmerdiven GE, Ak S, Ener B, Ağca H, Cilo BD, Tunca B, Akalın H. First Determination of Azole Resistance in Aspergillus fumigatus strains Carrying The TR34/L98H mutations in Turkey. J Infect Chemother. 2015;21(8):581-586. doi: 10.1016/j.jiac.2015.04.012.

[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

CD4+T-cell counts. *Indian J Med Microbiol*. 2018;36(3):408-415. doi: 10.4103/ijmm.IJMM_18_258.

[34] Herbrecht R, Denning DW, Patterson TF, Bennett JE, Greene RE, Oestmann JW, Kern WV, Marr KA, Ribaud P, Lortholary O, Sylvester R, Rubin RH, Wingard JR, Stark P, Durand C, Caillot D, Thiel E, Chandrasekar PH, Hodges MR, Schlamm HT, Troke PF, de Pauw B. Invasive Fungal Infections Group of the European Organisation for Research and Treatment of Cancer and the Global Aspergillus Study Group. Voriconazole versus Amphotericin B for Primary Therapy of Invasive Aspergillosis. *N Engl J Med*. 2002;347(6):408-415. doi: 10.1056/NEJMoa020191.

TABLES AND FIGURES WITH LEGENDS

TABLE 1. Demographic Characteristics of the Patients

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

AML: Acute Myeloid Leukemia. MM: Multiple Myeloma. NHL: Non-Hodgkin Lymphoma CLL: Chronic Lymphocytic Leukemia. ALL: Acute Lymphocytic Leukemia. HL: Hodgkin's Lymphoma. CML: Chronic Myeloid Leukemia. HCL: Hairy Cell Leukemia. Solid organ malignancy: Solid organ malignancy (such as lung and gynaecological cancers), Ewig's sarcoma, Metastatic tumor. Other hematological disease: Hemophagocytic syndrome, Undiagnosed cause unknown leukocytosis and leukopenia , Idiopathic thrombocytopenic purpura, Polycythemia vera, Myelodysplastic syndrome. Anemia: Iron deficiency anemia, Anemia, Aplastic anemia, Hemolytic anemia, Megaloblastic anemia.

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

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

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

Variable	IPA cases (n=56)	Non IPA cases (n=310)
GM positive results	44 (100%)	84 (100%)
ODI ≥ 0.5	16 (36.4%)	45 (53.6%)
ODI ≥ 1.0	11 (25.0%)	14 (16.7%)
ODI ≥ 1.5	2 (4.5%)	6 (7.1%)
ODI ≥ 2.0	15 (34.1%)	19 (22.6%)

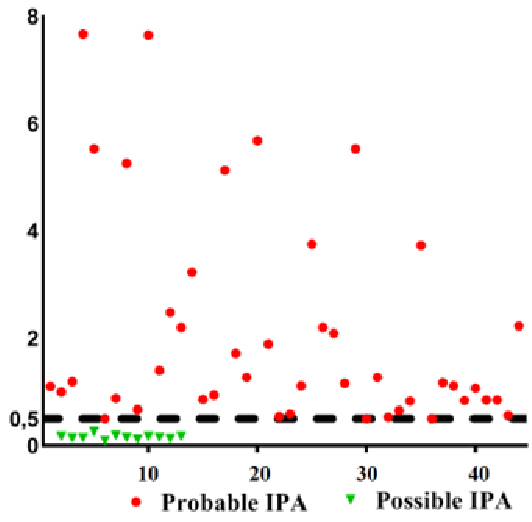

FIGURE 1. Scatter diagram displaying the GM ODI value of patients diagnosed with IPA

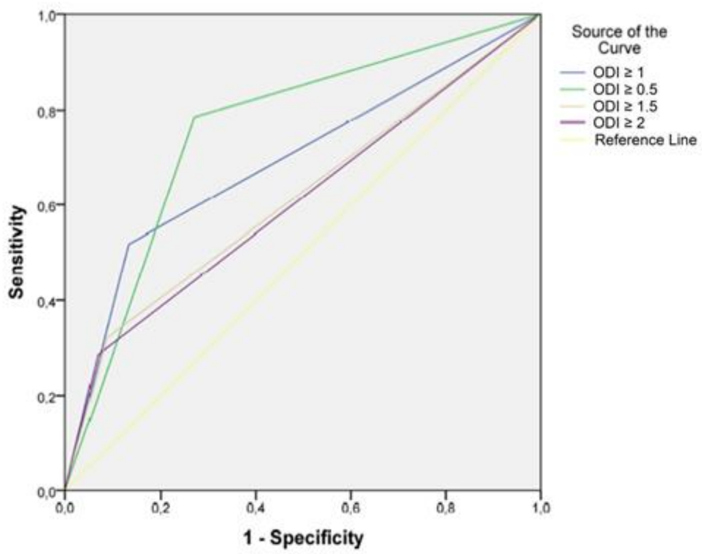

FIGURE 2. ROC curve for serum GM detection in patients with IPA