This article is cited as: Maçin S., Samadzade, R., Yılmaz, S., Fındık, D. (2024). Examination of Galactomannan Antigen Test Results in the Diagnosis of Patients with Suspect of Invasive Aspergillosis, *Mantar Dergisi*, 15(Special issue) 83-90.

Geliş(Recevied) :14.10.2024 ***Research Article***
Kabul(Accepted) :03.12.2024 **Doi: 10.30708/mantar.1566653**

Examination of Galactomannan Antigen Test Results in the Diagnosis of Patients with Suspect of Invasive Aspergillosis

Salih MAÇİN[1], Rugıyya SAMADZADE[2*], Senanur YILMAZ[3], Duygu FINDIK[4]

**Corresponding author: rukiyesamadzade@gmail.com*

[1] Selcuk University Faculty of Medicine, Department of Medical Microbiology, Konya, Türkiye/ salihmacin@hotmail.com
[2] Selcuk University Faculty of Medicine, Department of Medical Microbiology, Konya, Türkiye/ rukiyesamadzade@gmail.com
[3] Selcuk University Faculty of Medicine, Department of Medical Microbiology, Konya, Türkiye/ senanur.aydogan@outlook.com
[4] Selcuk University Faculty of Medicine, Department of Medical Microbiology, Konya, Türkiye/ dfindik@selcuk.edu.tr

Abstract: Invasive aspergillosis is an infectious disease caused by fungi from the Aspergillus species, and it typically has a high mortality rate among immunosuppressed individuals. Galactomannan antigen (GM) is a polysaccharide found in the cell wall of Aspergillus species and is used for early diagnosis, as traditional diagnostic methods often lead to significant delays in treatment. This study aimed to evaluate the results of galactomannan antigen testing in patients suspected of having invasive aspergillosis. Serum and bronchoalveolar lavage samples from patients with a preliminary diagnosis of invasive aspergillosis were sent to the laboratory for GM antigen testing. The GM antigens were detected using the Magnetic Bead-Coated Chemiluminescence method on the FACIS-I device. Galactomannan antigen positivity was found in 867 (15.3%) of the patients, with 492 (56.7%) of the positive samples coming from blood and 375 (43.3%) from bronchoalveolar lavage. Of the patients with positive galactomannan results, 282 (32.6%) were diagnosed with leukemia, 167 (19.2%) with pneumonia, and 142 (16.3%) with lymphoma. Among the samples that tested positive for the antigen, 343 (39.5%) were sent from Hematology, 292 (33.6%) from Chest Diseases, and 156 (18.1%) from Internal Medicine units. In conclusion, measuring galactomannan levels in serum or bronchoalveolar lavage samples can aid in the early diagnosis and treatment of invasive aspergillosis. Therefore, evaluating galactomannan test results may help reduce mortality and morbidity associated with invasive aspergillosis infections.

Keywords: Bronchoalveolar lavage, Galactomannan Antigen Test, Invasive Aspergillosis, Magnetic Bead Coated Chemiluminescence, Serum.

İnvaziv Aspergilloz Şüphesi Olan Hastaların Tanısında Galaktomanan Antijen Test Sonuçlarının Araştırılması

Öz: Genellikle immünsüpresif bireylerde yüksek mortalite ile seyreden invaziv aspergilloz, *Aspergillus* türü mantarların sebep olduğu enfeksiyon hastalıktır. Hastalığın klasik tanı yöntemlerinin geç sonuç vermesi tedavide önemli gecikmelere neden olduğu için erken tanıda

kullanılan galaktomannan antijeni, *Aspergillus* türünün hücre duvarında bulunan bir polisakkarittir. Bu çalışmanın amaçı invaziv aspergilloz şüphesi olan hastalarda galaktomannan antijen testinin sonuçlarının değerlendirilmesidir. Çalışmaya invaziv aspergilloz ön tanılı hastalardan galaktomannan antijeni isteğiyle laboratuvara gönderilen serum ve bronkoalveolar lavaj örnekleri dahil edilmiştir. Galaktomannan antijenlerinin tespiti, FACIS-I cihazında Manyetik Boncuk Kaplı Kemilüminesans yöntemi kullanılarak yapılmıştır. Hastaların 867'sinde (%15.3) galaktomannan antijeni pozitifliği saptanmışdır. Pozitif örneklerin 492'si (%56.7) kan, 375 'si (%43.3) bronkoalveolar lavaj olmuştur. Galaktomannan pozitifliği olan hastaların; 282'si (%32.6) lösemi, 167'si (%19.2) pnömoni ve 142'si (%16.3) lenfoma tanısı almıştır. Antijen pozitifliği saptanan örneklerin 343'ü (%39.5) Hematoloji, 292'si (%33.6) Göğüs hastalıkları ve 156'sı (%18,1)'si İç Hastalıkları ünitelerinden gönderilmiştir. Sonuç olarak serum veya bronkoalveolar lavaj örneklerinde galaktomannan düzeylerinin bakılmasının invaziv aspergillozun erken tanı ve tedavisinde yararlı olacağı kanısına varılmıştır. Bu nedenle galaktomannan test sonuçlarının klinik verilerle beraber değerlendirilmesi invaziv aspergilloz enfeksiyonlarında mortalite ve morbiditenin azaltılmasına katkı sağlayacaktır.

Anahtar Kelimeler: Bronkoalveolar lavaj, Galaktomannan Antijen Testi, İnvaziv Aspergillozis, Manyetik Boncuk Kaplı Kemilüminesans, Serum.

Introduction

One of the most important causes of mortality and morbidity in immunosuppressed individuals is invasive fungal infections (IFI). Cytotoxic chemotherapy agents and corticosteroids, especially those used in of treating immunosuppressed individuals, increase the prevalence of IFI infections (Kontoyiannis et al., 2010). In recent years, the frequency of invasive aspergillosis (IA) infections among immunocompromised patients and IFI's has been increasing. IA is mainly seen in immunocompromised patients with neutropenic fever who have undergone bone marrow transplantation and haematological malignancies, but has recently been reported in immunocompromised individuals (Ye et al., 2021).

IA infections are usually diagnosed by evaluating clinical findings and laboratory results together. The definitive diagnosis of the disease is made by direct microscopy and histopathological imaging of the agent in the clinical sample and identification at the species level achieved by growing in culture. However, in immunosuppressed patients, especially those with haematological malignancies, biopsy or deep tissue sampling is usually not possible impossible due to thrombocytopenia, hypoxia, and general deterioration (Schweer et al., 2014). Although findings supporting the diagnosis (such as halo, ground glass, for nodule) are obtained with radiological methods, these are not specific to IA (Denning et al., 2016). The search for new diagnostic methods has come to the forefront because it is difficult to obtain tissue samples in IA cases due to the general condition of the patients, the sensitivity and specificity of other clinical samples (sputum, blood, etc.) are insufficient in diagnosis, and it takes a long time to complete (Ray et al., 2022).

In recent years, faster and noninvasive tests have been developed to detect fungal DNA and serum antigen levels in of diagnosing IA. Serological tests, provide early and accurate diagnosis of critically ill patients (Lai et al., 2020). The first test used for antigen detection is the detection of galactomannan (GM) antigen, the exoantigen of *Aspergillus* species, in blood and body fluids. GM antigen levels can be detected in serum in the early stages of invasive aspergillosis before clinical symptoms occur. Detection of galactomannan antigen is important for early diagnosis and treatment in high-risk patients for invasive aspergillosis (Chowdhury et al., 2023). GM testing is included among the microbiological diagnostic criteria for of diagnosing invasive aspergillosis defined by the European Organization for Research and Treatment of Cancer-Mycosis Working Group (EORTC- MSG) (Bassetti et al., 2021). In addition, the GM test is a noninvasive diagnostic method approved by the Food and Drug Administration (FDA) for serum and BAL samples in 2003 and 2011 (Sav et al., 2014).

This study aimed to evaluate the GM antigen test results in serum and Bronchoalveolar lavage (BAL) samples of patients with a preliminary diagnosis of IA. Additionally, patients with GM positivity were analyzed according to gender, clinical sample, clinical diagnosis, and clinical units.

Material and Metod

Serum and BAL samples of patients with a preliminary diagnosis of IA, sent to the Medical Microbiology Laboratory of Selcuk University Faculty of Medicine between January 2020 and July 2024, with a

GM antigen request, were included in this study. Detection of GM antigens is performed by the Magnetic Bead Coated Chemiluminescence method using horseradish peroxidase horseradish peroxidase (HRP)-labelled anti-Aspergillus galactomannan antibodies on the FACIS-I instrument with the Fully Automated Chemiluminescence Immunoassay System (Genobio Pharmaceutical China).

Following the user manual of the FACIS-I device, the device was first logged in via the laboratory user system, then the controls were given and check-in was performed. Then, the sealing film for the user sample (sample treatment solution) and tip of the reagent strips were opened in accordance with the number of patients to be studied loaded into the device and scanned. Afterwards, the lot number of the kit used was checked and the barcode numbers of the patients were identified by placing them in the scanning area of the device. After the identification process was completed, 300 microliters of both serum and BAL samples were taken and added to the reagent strips and pipetted. After the samples were loaded into the device, the reagent strips were covered with air filters and the study was started. Regardless of the sample size, the study continued for 60 minutes. When interpreting the results, a cut-off of ≥ 0.5 in serum samples and ≥ 1 in BAL samples were considered positive. The results obtained were investigated retrospectively through hospital automation according to positivity, gender, clinical diagnosis and clinical unit criteria.

Results

A total of 5645 patients with a preliminary diagnosis of IA were included. GM antigen positivity was detected in 867 (15.3%) of these patients. Positive patients, 462 (53.3%) were male and 405 (46.7%) were female.

Antigen positivity was detected in 492 (56.7%) of blood samples and 375 (43.3%) of BAL samples (Figure 1).

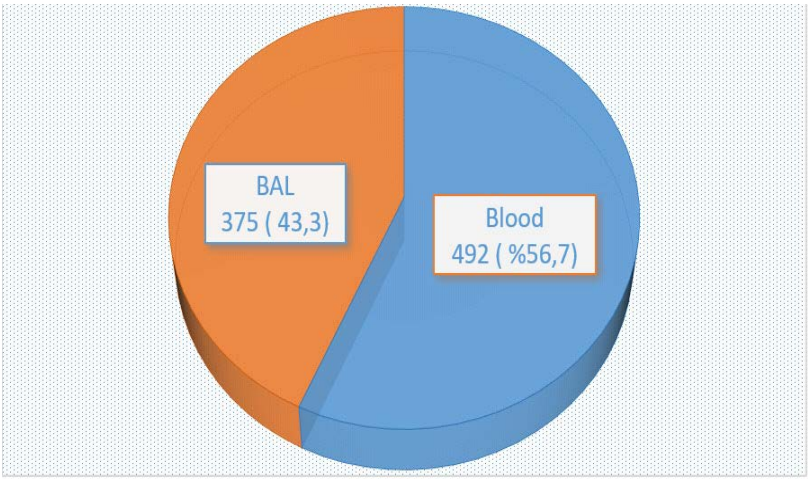

Figure 1. Distribution of positivity among samples

When the age distribution of the patients was examined, the highest positivity was found in patients over 60 years of age (47.5), and the lowest positivity was found in patients under 18 years of age (5.0). The distribution of GM positivity according to age groups is given in Table 1. Of the patients with GM positivity, 282 (32.6%) had leukaemia, 167 (19.2%) had pneumonia, 142 (16.3%) had lymphoma, 97 (11.2%) had various malignancies, 77 (19.2%) had acute bronchitis or bronchiectasis, 54 (6.2%) had various lung diseases, and 48 (5.6%) had other immunodeficiencies (Table 2.). Of the patient samples with antigen positivity, 343 (39.5%) were sent from the Hematology Unit, 292 (33.6%) from the Chest Diseases Unit, 156 (18.1%) from the Internal Medicine Unit, 51 (5.9%) from the Anesthesia and Reanimation Unit, and 25 (2.9%) from the Infectious Diseases Unit (Table 3.).

Table 1. Distribution of of patients with GM positivity by age groups

Age Groups	Positivity n (%)
≤18	412 (47.5)
19-35	311 (35.9)
36-60	101 (11.6)
>60	43 (5.0)

Table 2. Distribution of patients with GM positivity according to clinical diagnosis

Clinical Diagnoses	Positivity n (%)
Leukemia	282 (32.6)
Pneumonia	167 (19.2)
Lymphoma	142 (16.3)
Various Malignancies*	97 (11.2)
Bronchitis or Bronchiectasis	77 (8.9)
Various Lung Diseases**	54 (6.2)
Other Immunodeficiencies***	48 (5.6)

*Colon, larynx, lung, soft tissue, brain, renal, prostate and gall bladder malignancies
**Tuberculosis, Candidiasis, Pulmonary Embolism, Chronic obstructive pulmonary disease, COVID-19
***Acute Renal Failure, Ulcerative Colitis, Crohn's Disease, Chronic Viral Hepatitis B, Iron Deficiency Anemia, Idiopathic Thrombocytopenic Purpura

Table 3. Distribution of patients with GM positivity according to clinical units

Clinical Units	Positivity n (%)
Hemotology Unit	343 (39.5)
Chest Diseases Unit	292 (33.6)
Internal Medicine Units*	156 (18.1)
Pediatrics Units**	84 (9.6)
Anesthesia and Reanimation Unit	51 (5.9)
Infectious Diseases Unit	25 (2. 9)

*Medical Oncology, Rheumatology, Gastroenterology
**Oncology, Intensive Care, Rheumatology, Gastroenterology

Discussions

IA usually develops in immunosuppressed patients and causes increased mortality and morbidity. IA is diagnosed by evaluating clinical findings, radiological and laboratory data together. However, since clinical symptoms and radiological findings specific to IA are often not detected or are not specific, they can be confused with other diseases. Therefore, early clinical and radiological diagnosis is not possible (Kono et al., 2013). Classical methods such culture and direct microscopy methods used in the microbiological diagnosis of IA are the gold standards and still maintain their importance in diagnosis. In addition, invasive diagnostic methods cannot be performed adequately when hemostasis defects such as neutropenia and thrombocytopenia occur in patients with hematological malignancies in laboratory diagnosis (Guegan et al., 2018). Both the long waiting time for culture results and other difficulties in diagnosis cause significant delays in treatment, increasing mortality and morbidity rates. Therefore, early diagnosis is important to start appropriate treatment as soon as possible (De Heer et al., 2019).

Recently, GM antigen test, which is easier, faster and gives earlier results than conventional methods, has been used in routine diagnosis. The presence of GM antigen was examined using many samples such as cerebrospinal fluid, serum, pleural fluid, BAL, and lung tissue. (Sav et al., 2014). Tanase et al. (2012), reported the sensitivity and specificity, positive predictive value (PPV) and negative predictive value (NPV) of the serum GM test (cut-off 0.5 ng/mL) as 85%, 91%, 46% and 99%, respectively (Tanase et al., 2012). Lehrnbecher et al. (2012) showed in their meta-analysis that GM negative predictive values are high, ranging from 85% to 100% for screening and 70% to 100% in the diagnostic setting. It was emphasized that non-Aspergillus fungi should be considered as the cause of false positivity (Lehrnbecher et al., 2012).

Haydour et al. (2019), conducted a systematic review and meta-analysis of studies evaluating the diagnostic accuracy of serum and BAL GM, in suspected patients, in critically ill patients at risk for

candidiasis or candidemia. In conclusion, in immunocompromised patients with suspected IA, serum GM had a sensitivity of 0.71 (95% confidence interval [CI], 0.64-0.78) and a specificity of 0.89 (95% CI, 0.84-0.92). The optical density index cut- off value provided optimal sensitivity and specificity. In immunocompromised patients with suspected IA, BAL GM had a sensitivity of 0.84 (95% CI, 0.73-0.91) and a specificity of 0.88 (95% CI, 0.81-0.91). In immunocompromised patients with suspected IA, serum or whole blood PCR had a sensitivity of 0.81 (95% CI, 0.73-0.86) and a specificity of 0.79 (95% CI, 0.68-0.86). In patients at high risk of IA, BAL PCR had a sensitivity of 0.90 (95% CI, 0.77-0.96) and a specificity of 0.96 (95% CI, 0.93-0.98) for the diagnosis of IA. These results show that the diagnosis of IA infections is still difficult and the combined use of serum and BAL markers may help in accurate diagnosis (Haydour et al., 2019).

Sav et al. (2014), studied the GM test (Platelia Aspergillus EIA; Bio-Rad) and β-D glucan (BG) test (Fungitell, Associates of Cape Cod, USA) in serum samples taken from patients with IA diagnosis (n=42) and the control group (n=37). The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the BG test (cut-off 80 pg/mL) for the diagnosis of IA were found to be 78%, 78%, 80%, and 76%, respectively. GM test was performed on the same serum samples and the sensitivity, specificity, PPD, and NPD values of the test were determined as 47%, 89%, 83%, and 60%, respectively (cut-off 0.5 ng/mL). As a result, it was determined that the BG test had the highest sensitivity and the GM test had the highest specificity, and it was reported that these tests should be evaluated together with clinical and radiological findings to increase the significance of non-culture methods in the diagnosis of IA (Sav et al., 2014).

Aslan et al. (2016), detected GM Ag positivity at least twice in a row in 10 of 39 patients with clinical and/or radiological findings in terms of IA followed up with the diagnosis of multiple myeloma, according to the European Organisation for Research and Treatment of Cancer-Mycosis Working Group (EORTC-MSG) guideline. However, with Real-time PCR, Aspergillus DNA was found negative in all serum samples of 39 multiple myeloma patients with IA and 29 control patients. Therefore, although the GM Ag test is a useful non-invasive test in the early diagnosis of IA, it should not be forgotten that false-positive results can be obtained in many cases and should be evaluated together with other clinical and laboratory test results (Aslan et al., 2016).

False positivity of the GM test poses a significant problem in diagnosis. False positive results of GM tests can be caused by many factors. The main reason for this is that GM is found in many bacteria and fungi. (Oz and Kiraz, 2011). False-positive results in GM tests have been associated with epidemiological and biological factors such as cross-reactivity with GM from other fungi (*Blastomyces*, *Histoplasma*, *Cryptococcus, Penicillium*, *Fusarium*, *Cladosporium*, *Paracoccidioides*, *Nigrospora*, *Paecilomyces*, *Geotrichum*, *Trichothecium* and *Lichtheimia ramosa*) and with some beta-lactam antibiotics (piperacillin-tazobactam, amoxicillin- clavulanate). This is because galactomannan from bacteria and foods with cross-reactive epitopes can be transported across the intestinal mucosa during periods when mucosal integrity is disrupted (Mennink-Kersten et al., 2005).

In two similar studies, it was found that antifungal treatment effective against mold in patients receiving posaconazole or voriconazole prophylaxis prevented GM circulation in the blood and decreased the sensitivity of serum GM detection in concomitantly applied tests (Cornely, 2014; Duarte et al., 2014). Vena et al. (2017), the performance of routine serum GM in the diagnosis of IA was investigated by in high-risk hematology patients receiving micafungin prophylaxis. According to the results, it was reported that the use of GM in the follow- up of asymptomatic patients receiving micafungin prophylaxis was unnecessary because the results were negative or false positive in consecutive samples. Therefore, GM testing was recommended for the diagnosis of sudden IA in symptomatic patients during prophylaxis (Vena et al., 2017). In a retrospective pediatric study by Limber et al. (2011), the sensitivity of the GM serum test was reported as 0.91 and the specificity as 0.81. The false positive rate was found to be 18.3%. Suggested that this may be due to the different levels of GM production ability in some Aspergillus species. In addition, it has been suggested that early initiation of antifungal therapy may also lead to false- negative results (Limber et al., 2011).

As a result, it was concluded that checking GM levels in consecutive serum or BAL samples would be useful in the early diagnosis and treatment of IA infections. In addition, it is very important to evaluate test results in cooperation between the laboratory and the clinician. Because GM test results are very helpful in the diagnosis of IA when they are consistent with clinical data. Therefore, evaluating GM test results in accordance with clinical data and using them at appropriate frequency in serological diagnosis will contribute to reducing mortality and morbidity of IA infections.

Author contributions

All authors have equal contributions.

Conflicts of interest

The authors declare no competing interests.

Ethical Statement: It is declared that scientific and ethical principles have been followed while carrying out and writing this study and that all the sources used have been properly cited (Salih MAÇİN, Rugıyya SAMADZADE, Senanur YILMAZ, Duygu FINDIK).

Acknowledgement

We would like to thank the entire research team for their contributions in preparing the study in line with the principles of high ethics, scientificness, honesty and openness.

References

Aslan, M., Öz, Y., Akşit, F., Akay M.O., and Aslan, F.K. (2016). Does Multıple Myeloma cause false posıtıve results of the *Aspergillus galactomannan* assay? *O.J.M*, 38 (2), 47-50.

Bassetti, M., Azoulay, E., Kullberg, B.J., Ruhnke M., Shoham, S., Vazquez, J., Giacobbe, D.R., and Calandra, T.(2021). EORTC/MSGERC definitions of ınvasive fungal diseases: summary of activities of the ıntensive care unit working group. *Clin. Infect. Dis*, 72(2), S121-S127.

Chowdhury T.C., M., Singh, G., Pandey, M., Mishra, H., Meena, V.P., Sethi, P., Singh, A., Prakash, B., Upadhyay, A.D., Mohan, A., Sinha, S., Xess, I., Wig, N., Kabra, S.K., and Ray, A. (2023). The utility of galactomannan and polymerase chain reaction assays in Bronchoalveolar lavage for diagnosis of chronic pulmonary Aspergillosis. *Mycopathologia*, 188(6), 1041-1053.

Cornely, O.A. (2014). Galactomannan testing during mold-active prophylaxis. *Clin. Infect. Dis*, 59 (12), 1703-1704.

De Heer, K., Gerritsen, M.G., Visser, C.E., and Leeflang, M.M. (2019). Galactomannan detection in broncho-alveolar lavage fluid for invasive aspergillosis in immunocompromised patients. Cochrane. *Database. Syst. Rev*, 5(5):CD012399.

Denning, D.W., Cadranel, J., Beigelman-Aubry, C., Ader, F., Chakrabarti, A., Blot, S., Ullmann, A.J., Dimopoulos, G., Lange, C., and European Society for Clinical Microbiology and Infectious Diseases and European Respiratory Society. (2016). Chronic pulmonary aspergillosis: rationale and clinical guidelines for diagnosis and management. *Eur. Respir. J*, 47(1), 45-68.

Duarte, R.F., Sánchez-Ortega, I., Cuesta, I., Arnan M., Patino B., Fernández de Sevilla A., Gudiol C., Ayats J., and Cuenca-Estrella, M. (2014). Serum galactomannanbased early detection of invasive aspergillosis in hematology patients receiving effective antimold prophylaxis. *Clin. Infect. Dis*, 59 (12), 1696-1702.

Guegan, H., Robert-Gangneux, F., Camus, C., Belaz, S., Marchand, T., Baldeyrou, M., and Gangneux, J.P. (2018). Improving the diagnosis of invasive aspergillosis by the detection of Aspergillus in broncho-alveolar lavage fluid: comparison of non-culture-based assays. *J. Infect*, 76(2), 196-220.

Haydour, Q., Hage, C.A., Carmona, E.M., Epelbaum. O., Evans S.E., Gabe, L.M., Knox, K.S., Kolls, J.K, Wengenack, N.L., Prokop, L.J., Limper, A.H., and Murad, M.H.(2019). Diagnosis of fungal infections. A systematic review and meta-analysis supporting american thoracic society practice guideline. *Ann. Am. Thorac. Soc*, 16(9),1179-1188.

Kono, Y., Tsushima, K., Yamaguchi, K., Kurita, N., Soeda, S., Fujiwara, A., Sugiyama, S., Togashi, Y., Kasagi, S., To, M., To, Y., and Setoguchi, Y. (2013). The utility of galactomannan antigen in the bronchial washing and serum for diagnosing pulmonary aspergillosis. *Respir. Med*, 107, 1094-1100.

Kontoyiannis, D.P., Marr, K.A., Park, B.J., Alexander, B.D., Anaissie, E.J., Walsh, T.J., Ito, J., Andes,D.R., Baddley,J.W., Brown, J.M., Brumble, L.M., Freifeld,A.G., Hadley, S., Herwaldt, L.A., Kauffman,C.A., Knapp, K., Lyon, G.M., Morrison, V.A., Papanicolaou, G.,Patterson, T.F., Perl,T.M., Schuster,M.G., Walker, R., Wannemuehler, K.A., Wingard, J.R., Chiller, T.M., and Pappas, P.G.(2010). Prospective surveillance for invasive fungal infections in hematopoietic stem cell transplant recipients, 2001-2006: overview of the Transplant-Associated Infection Surveillance Network (TRANSNET) Database. *Clin. Infect. Dis*, 50(8), 1091-1100.

Lai, G., Zeng, C., Mo, J., Song, W.D., and Xu, P. (2020). Diagnostic value of galactomannan in Bronchoalveolar lavage fluid for chronic respiratory disease with pulmonary Aspergillosis. *J. Clin. Microbiol*, 58, e01308-e01319.

Lehrnbecher, T., Phillips, R., Alexander, S., Alvaro, F., Carlesse, F., Fisher, B., Hakim, H., Santolaya, M., Castagnola, E., Davis, B.L., Dupuis,L.L., Gibson, F., Groll A.H., Gaur, A., Gupta, A., Kebudi, R., Petrilli, S., Steinbach, W.J., Villarroel, M., Zaoutis, T., Sung, L., and International Pediatric Fever and Neutropenia Guideline Panel. (2012). Guideline for the management of fever and neutropenia in children with cancer and/ or undergoing hematopoietic stem-cell transplantation. *J. Clin. Oncol*, 30: 4427–4438.

Limper, A.H., Knox, K.S., Sarosi, G.A., Ampel, N.M., Bennett, J.E., Catanzaro, A., Davies, S.F., Dismukes, W.E., Hage, C.A., Marr, K.A., Mody, C.H., Perfect, J.R., Stevens, D.A., and American Thoracic Society Fungal Working Group. (2011). An official American Thoracic Society statement: Treatment of fungal infections in adult pulmonary and critical care patients. *Am. J. Respir. Crit. Care. Med*, 183 (1), 96-128.

Mennink-Kersten, M.A., Ruegebrink, D., Klont, R.R., Warris, A., Gavini, F., Op den Camp, H.J.M., and Verweij, P.E. (2005). Bifidobacterial lipoglycan as a new cause for false-positive Platelia Aspergillus enzyme-linked immunosorbent assay reactivity. *J. Clin. Microbiol*, 43 (8), 3925–3931.

Oz, Y., and Kiraz, N. (2011). Diagnostic methods for fungal infections in pediatric patients: microbiological, serological and molecular methods. *Expert. Rev. Anti. Infect. Ther*, 9(3), 289-298.

Ray, A., Chowdhury, M., Sachdev, J., Sethi, P., Meena, V.P., Singh, G., Xess, I., Vyas, S., Khan, M.A., Sinha, S., Denning, D.W., Wig, N., and Kabra, S.K. (2022). Efficacy of LD Bio Aspergillus ICT Lateral Flow Assay for serodiagnosis of Chronic Pulmonary Aspergillosis. *J. Fungi*, 8(4), 400.

Sav, H., Koç, N., Atalay, M.A., Yıldız, O., and Demir, G. (2014). Investigation of various methods in the diagnosis of systemic aspergillosis. *Flora* ,19(2), 66-73.

Schweer, K.E., Bangard, C., Hekmat, K., and Cornely, O.A. (2014). Chronic pulmonary aspergillosis. *Mycoses*, 57(5), 257-270.

Tanase, A.D., Colita, A., Marculescu, A., Berteanu, C., Streinu, Cercel, A., Stoica, M., Stoica, A., Cernea, D., Copotoiu, S., Brînzaniuc, K., and Azamfirei, L. (2012). Using the galactomannan antigen assay in the diagnosis of invasive aspergillosis after hematopoietic stem cell transplantation. *Rom. J. Morphol. Embryol*, 53:379-382.

Vena, A., Bouza, E., Álvarez-Uría, A., Gayoso, J., Martín-Rabadán, P., Cajuste, F., Guinea, J., Gómez Castellá, J., Alonso, R., and Munoz, P. (2017). The misleading effect of serum galactomannan testing in high-risk haematology patients receiving prophylaxis with micafungin. *Clin. Microbiol. Infect*, 23(12),1000.e1-1000.e4.

Ye, F., Zeng, P., Li, Z., Tang, C., Liu, W., Su, D., Zhan, Y., and Li, S. (2021). Detection of Aspergillus DNA in BALF by real-time PCR and galactomannan antigen for the early diagnosis of chronic pulmonary Aspergillosis. *Ann. Clin. Lab. Sci*, 51(5), 698-704.