Erciyes Med J 2018; 40(2): 57-60 • DOI: 10.5152/etd.2018.0069

Galactomannan Antigen Detection as a Screening Tool For Early Diagnosis of Invasive Aspergillosis: Experience of Turkish Adult Patients

INVITED REVIEW

Gökhan Metan[1], Hamdi Akan[2]

ABSTRACT

Objective: The aim of the present study was to investigate the usefulness of galactomannan (GM) antigen as a screening test for the early diagnosis of invasive aspergillosis (IA) in adult patients in Turkish centers.

Materials and Methods: PubMed was searched using the keywords "galactomannan" and "Turkey." Only studies that used the GM antigen as a screening tool in adult patients were included in the analysis.

Results: Four peer-reviewed articles that matched the inclusion criteria were identified. A total of 314 adult patients with several hematological malignancies and who underwent allogeneic hematopoietic stem cell transplantation were included in the four studies. Patients were followed up for 459 neutropenia episodes. GM antigen testing was performed in 2662 serum samples. The sensitivity, specificity, positive predictive value, and negative predictive value were 23.07%–100%, 5.7%–90.36%, 6.7%–73.07%, and 55.5%–100%, respectively. Early diagnosis of IA by GM screening was found in only one patient in one of the four studies.

Conclusion: The performance of the GM antigen test as a screening tool in Turkish centers was not promising. However, it can be used as a diagnostic test for patients with clinical and radiological findings suggesting invasive fungal disease.

Keywords: Galactomannan, invasive aspergillosis, diagnosis, Turkey

Cite this article as: Metan G, Akan H. Galactomannan Antigen Detection as a Screening Tool For Early Diagnosis of Invasive Aspergillosis: Experience of Turkish Adult Patients. Erciyes Med J 2018; 40(2): 57-60.

[1]Department of Infectious Diseases and Clinical Microbiology, Hacettepe University Faculty of Medicine, Ankara, Turkey
[2]Department of Hematology, Ankara University Faculty of Medicine, Ankara, Turkey

Submitted 02.05.2018

Accepted 03.05.2018

Correspondence Gökhan Metan, Department of Infectious Diseases and Clinical Microbiology, Hacettepe University Faculty of Medicine, Ankara, Turkey
Phone: 90 312 305 1296
e.mail: gokhanmetan@gmail.com, gokhanmetan@hacettepe.edu.tr

INTRODUCTION

The diagnosis of invasive aspergillosis (IA) is a challenge in patients with hematological malignancies. Although isolation of *Aspergillus* species from fungal culture is the gold standard, several conditions such as severe thrombocytopenia and hypoxemia can limit to obtain deep tissue or bronchoalveolar lavage fluid to perform fungal culture. The low sensitivity of clinical and radiological signs can cause a significant delay in the accurate diagnosis of IA (1).

Detection of *Aspergillus* galactomannan (GM) antigen in serum by sandwich enzyme immunoassay method (Bio-Rad Laboratories, Marnes-la-Coquette, France) has been accepted as an important tool for the early diagnosis of IA. Although screening of high-risk hematology patients with the GM antigen was recommended in several guidelines, the performance of the test can be influenced by several factors such as duration of neutropenia, number of neutrophils, sampling schedule, incidence of IA, exposure to mold-active antifungal drugs, and laboratory experience (1, 2). The Turkish national expert report for the diagnosis of invasive fungal diseases (IFDs) recommended the GM antigen as a screening test based on the studies performed in different countries (3). There was only one study from Turkey regarding the GM antigen detection in high-risk hematology patients for the early diagnosis of IA when this report was written (4). Therefore, no country-specific recommendation could be made in the national expert report (3). The aim of the present study was to review the recently published national literature and to evaluate the role of GM antigen screening for the early diagnosis of IA in Turkish centers.

MATERIALS and METHODS

PubMed was searched using the keywords "galactomannan" and "Turkey." Only studies that investigated the performance of the GM antigen in adult patients as a screening test were included in the literature analysis.

RESULTS

Four peer-reviewed articles from Turkey about the diagnostic performance of the GM antigen as a screening test for the diagnosis of IA were published at international journals between 2010 and 2018 and all of them included adult patients with different hematological malignancies or hematopoietic stem cell transplantation (HSCT) (4-7). Twice weekly sampling was performed in all studies. Screening tests were started at the day of an absolute neutrophil count

Table 1. Characteristics of the studies and diagnostic performance of galactomannan antigen

Study center Time period	Number of the patients	Number of the patients with IA Diagnostic criteria	Number of the serum samples	Antifungal prophylaxis	Cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Reference number
Hacettepe University 2001-2003	58 neutropenia episodes in 45 patients	1 proven 4 probable 20 possible EORTC/MSG 2002	545	None	Single positive results ODI ≥ 0.5 Two consecutive positive results ODI ≥ 0.5	100 60	5.7 20.8	9.1 6.7	100 84.6	4
Uludag University 2003-2004	165 neutropenia episodes in 106 patients	4 proven 11 probable 65 possible EORTC/MSG 2008	1385	Antifungal administration in 111 episodes, types of antifungal drugs were not stated	Single positive results ODI ≥ 0.5 Two consecutive positive results ODI ≥ 0.5	100 86.7	27.1 71.8	19.5 35.1	100 96.8	5
Osmangazi University 2008-2011	161 neutropenia episodes in 99 patients	1 proven 17 probable 60 possible EORTC/MSG 2008	358 from high risk patients and 20 from non-neutropenic patients without fever	Antifungal administration in 106 episodes, mold active drugs but exact types were not stated	Single positive results ODI ≥ 0.5	23.07	90.36	73.07	55.55	6
Erciyes University 2012-2013	75 neutropenia episodes in 64 patients	12 probable 1 possible EORTC/MSG 2008	354	Posaconazole in 31 episodes Fluconazole in 42 episodes Voriconazole in 2 episodes	Single positive results ODI ≥ 0.7	35.7	99.6	3.1	96.8	7

IA; invasive aspergillosis, EORTC/MSG; 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, PPV; positive predictive value, NPV; negative predictive value, ODI; optical density index

of <500/mm^3 until recovery of neutropenia except in Uludag University Hospital where GM screening started at the first day of hospitalization. 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) criteria published in 2002 was used in the study performed at Hacettepe University Hospital to define the probability of IA (4, 8). Other studies used the EORTC/MSG criteria published in 2008 to define IA (5-7, 9).

A total of 301 adult patients with several hematological malignancies and 13 patients who underwent allogeneic HSCT were included in the four studies. Patients were followed up for 459 neutropenia episodes. GM antigen testing was performed in 2662 serum samples. Mold-active antifungal prophylaxis was administered in two studies (6, 7). Fluconazole was the choice of antifungal prophylaxis in other studies (4, 5).

The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were 23.07%–100%, 5.7%–90.36%, 6.7%–73.07%, and 55.5%–100%, respectively (Table 1). Early diagnosis of IA by GM antigen screening was reported in only one patient (positive GM before detection of *Aspergillus* rhinosinusitis) in one of the four studies (4).

DISCUSSION

The diagnostic performance of GM was highly variable between the centers. The sensitivity of the GM antigen was low in the first study from Turkey (4). This can be associated with the high rate of false-positive GM related to piperacillin–tazobactam (TZP) administration in the same center (10). The study from Uludag University Hospital showed a better performance contrast to the Hacettepe University study (Table 1), but there were still 65 patients with clinical and radiological findings concordant with IA (possible cases based

on the EORTC/MSG criteria) in which the GM antigen was negative despite screening twice a week (4, 5). Both centers did not use mold-active prophylaxis during the study periods, and false-positive GM related to TZP administration was not an issue in Uludag University (11). The debate on false-positive GM related to the administration of TZP (original brand) appeared to be resolved in Turkish centers, but false-positive GM in patients receiving a generic TZP was reported very recently (12-14). Obtaining the blood sample for GM antigen test just before the next dose of TZP was shown to be a very effective way for avoiding false positivity (15).

Previous studies reported very low sensitivity (23.07%–35.7%) for GM screening that was performed when posaconazole prophylaxis became the standard of care particularly for patients receiving induction chemotherapy for acute myeloid leukemia (AML) (6, 7). However, the specificity was higher than 90%, indicating that a positive GM result should trigger the diagnostic pathway for the early detection of IA (6, 7).

A total of 2972 serum GM tests were performed in a 4-year study with 262 unselected consecutive high-risk episodes that received posaconazole prophylaxis. GM antigen was negative in 96.7% of tests and 83.6% of episodes. There were 30 false-positive episodes that mainly occurred in tests performed as preemptive surveillance (26 out of these 30 episodes (86.7%)). When GM antigen testing was used for screening, PPV was 11.8%, whereas PPV increased to 89.6 when used a diagnostic test in case of suspicion of IFD. NPV remained at 100% at any scenario (16). While the sensitivity of GM in serum decreases under mold-active prophylaxis, GM antigen can remain positive in the bronchoalveolar lavage fluid despite posaconazole prophylaxis (17).

In a study from Erciyes University, the serum samples from patients with radiological evidence of IA and from patients without IA (1 to 10 ratio) were re-tested by a modified methodology that was previously proven to increase the sensitivity of GM antigen detection (7, 18). The sensitivity increased from 35.7% to 92.3% with a slight decrease in the specificity from 99.6% to 97.6% (7). The method is based on the concentration of GM antigen. It can be suitable for patients with radiological findings suggesting IA rather than a screening test due to high workload and extra cost.

Galactomannan (GM) antigen screening was supported as an early diagnostic tool for IA since 2001 and recommended by several guidelines as part of the routine care for patients with prolonged neutropenia (1, 2, 19). However, there are some studies not concordant with this statement. A previous study showed that GM antigen detection does not precede detection of major lesions by pulmonary computed tomography (CT) scan. The authors reported that only 7 (10%) out of 70 patients with pulmonary signs of IA have positive GM antigen test results before detection of the pathological change by CT scan (20). When the results of the randomized controlled study that compared voriconazole and amphotericin B in the treatment of IA were re-evaluated using the EORTC/MSG 2008 criteria, possible cases who received voriconazole had better outcomes than patients who received amphotericin B. The authors concluded that possible cases diagnosed by radiology without positive GM antigen or *Aspergillus* isolation are real aspergillosis diagnosed early by radiology (21). The rate of possible IFD (diagnosed by radiology without any microbiological evidence of IFD) was higher than that of probable IFD in the recent prospective European audit for invasive mold diseases that recruited patients with AML and allogeneic HSCT. Only centers that have an immediate access to GM antigen testing were allowed to enroll patients in the study, and it was surprising that possible cases were more frequent despite serial GM screening in a prospective study (22). These findings raise questions about GM antigen screening for the early diagnosis of IA. A recent study from Switzerland analyzed the performance of the GM antigen as a screening tool in patients with hematological cancer with duration of neutropenia longer than 14 days. Serum GM antigen was measured two times per week, and mold-active prophylaxis was not routinely part of the care. IA was diagnosed in 30 out of 268 patients, and a positive serum GM was the first indicator of IA in 10 (33%) patients. A total of 500 GM antigen tests were required to diagnose one IA case based on GM antigen screening, costing $15,000 in this setting (23). Cost and logistics are other important issues to use GM antigen screening as an effective tool for the early diagnosis of IA. The time period for reporting the results of GM antigen test was reported as 1 week from a Turkish university hospital (24).

There were also two pediatric studies investigating the role of GM antigen for the diagnosis of IA. The first study was from Ege University that included 141 patients with acute lymphoblastic leukemia followed up between 2006 and 2015. GM antigen was tested in 3264 serum samples. The authors did not calculate the sensitivity or specificity of GM antigen but investigated the role of GM antigen screening for the management of patients with neutropenia in detail. There were 5 patients with proven or probable IA. Of the 3264 serum samples, 179 (5.5%) from 76 patients were positive at a cut-off value of >0.5. Of the 76 patients, 21.7% were true positives, and 52.1% were false positives. Thorax CT scan revealed findings concordant with IA in 12 (15.8%) of the 76 patients with positive GM antigenemia. Of the 76 patients, 35 with positive GM antigen were already on mold-active antifungal therapy. The positive GM antigen results changed the antifungal treatment in only 13 (17.1%) patients (25). The second study included 47 hematological patients below 18 years old who had at least one GM antigen test result. A total of 158 blood samples were tested for GM antigen and yielded a sensitivity of 36% and a specificity of 90.9% at a cut-off value of 0.7. Decreasing the cut-off value for GM antigen to 0.5 increased the sensitivity to 68% but decreased the specificity to 77.2% (26).

CONCLUSION

In Turkey, access to GM antigen test is available in most of the centers treating high-risk hematology patients, but publication of the experience in the international literature is limited (4-7, 25-27). Only one center reported GM antigen as a useful marker for the diagnosis of IA where mold-active prophylaxis was not used (5). After posaconazole prophylaxis was introduced in the clinical practice, the sensitivity of GM antigen was low, and the antifungal therapy was mainly triggered by radiological investigation (7). In addition, the benefit of GM antigen screening was extremely limited in pediatric patients (25, 26).

In conclusion, the performance of the GM antigen test as a screening tool is inferior in Turkish centers based on the findings of the limited number of studies. However, retaining GM antigen as a diagnostic test for patients with clinical and radiological findings suggesting IA will be useful to achieve microbiological signs about the etiology of suspected IFD.

Peer-review: Externally peer-reviewed.

Author Contributions: Conceived and designed the review procudure: GM, HA. Performed the literature review from Pubmed: GM. Analyzed the data: GM, HA. Wrote the paper: GM, HA. All authors have read and approved the final manuscript.

Conflict of Interest: Authors have no conflicts of interest to declare.

Financial Disclosure: Authors declared that this study has received no financial support.

REFERENCES

1. Ullmann AJ, Aguado JM, Arikan-Akdagli S, Denning DW, Groll AH, Lagrou K, et al. Diagnosis and management of Aspergillus diseases: executive summary of the 2017 ESCMID-ECMM-ERS guideline. Clin Microbiol Infect 2018; 24 Suppl 1:e1-e38. [CrossRef]
2. Patterson TF, Thompson GR 3rd, Denning DW, Fishman JA, Hadley S, Herbrecht R, et al. Practice Guidelines for the Diagnosis and Management of Aspergillosis: 2016 Update by the Infectious Diseases Society of America. Clin Infect Dis 2016; 63(4):e1-e60. [CrossRef]
3. Arıkan Akdağlı S, Azap A, Başaran Demirkazık F, Ener B, Aşçıoğlu Hayran S, et al. Diagnosis of Invasive Fungal Diseases in Hematological Malignancies: A Critical Review of Evidence and Turkish Expert Opinion (TEO-2). Turk J Haematol 2014; 31(4):342-56. [CrossRef]
4. Tanriover MD, Ascioglu S, Altun B, Uzun O. Galactomannan on the stage: prospective evaluation of the applicability in routine practice and surveillance. Mycoses 2010; 53(1):16-25. [CrossRef]
5. Okuturlar Y, Ozkalemkas F, Ener B, Serin SO, Kazak E, et al. Serum galactomannan levels in the diagnosis of invasive aspergillosis. Korean J Intern Med 2015; 30(6):899-905. [CrossRef]
6. Aslan M, Oz Y, Aksit F, Akay OM. Potential of polymerase chain reaction and galactomannan for the diagnosis of invasive aspergillosis in patients with febrile neutropenia. Mycoses 2015; 58(6):343-9. [CrossRef]
7. Metan G, Keklik M, Dinc G, Pala C, Yıldırım A, Saraymen B, et al. Performance of Galactomannan antigen, beta-D-glucan, and Aspergillus-lateral-flow device for the diagnosis of invasive aspergillosis. Indian J Hematol Blood Transfus 2017; 33(1):87-92. [CrossRef]
8. Ascioglu S, Rex JH, Pauw B Bennett JE, Bille J, Crokaert F, et al; Invasive Fungal Infections Cooperative Group of the European Organization for Research and Treatment of Cancer; Mycoses Study Group of the National Institute of Allergy and Infectious Diseases. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants. An international consensus. Clin Infect Dis 2002; 34(1): 7–14. [CrossRef]
9. De Pauw B, Walsh TJ, Donnelly JP, Stevens DA, Edwards JE, Calandra T, et al; European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group.; National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. 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(12):1813-21. [CrossRef]
10. Tanriover MD, Metan G, Altun B, Hascelik G, Uzun O. False positivity for Aspergillus antigenemia related to the administration of piperacillin/tazobactam. Eur J Intern Med 2005; 16(7):489-91. [CrossRef]
11. Ozkalemkas F, Ozcelik T, Ozkocaman V. Treatment with piperacillin-tazobactam and Aspergillus galactomannan test results for patients with hematological malignancies. Eur J Intern Med 2007; 18(1):79. [CrossRef]
12. Yucesoy M, Ergon MC. Investigation of Aspergillus galactomannan levels in antimicrobial agents. Mikrobiyol Bul 2007; 41(4):565–70.
13. Metan G, Ağkuş C, Buldu H, Koç AN. The interaction between piperacillin/tazobactam and assays for Aspergillus galactomannan and 1,3-beta-D-glucan in patients without risk factors for invasive fungal infections. Infection 2010; 38(3):217-21. [CrossRef]
14. Demiraslan H, Atalay MA, Eren E, Demir K, Kaynar L, Koc AN, Doganay M. Assessing the risk of false positive serum galactomannan among patients receiving piperacillin/tazobactam for febrile neutropenia. Med Mycol. 2017 55(5):535-540.
15. Penack O, Rempf P, Graf B, Thiel E, Blau IW. False-positive Aspergillus antigen testing due to application of piperacillin/tazobactam–is it still an issue? Diagn Microbiol Infect Dis 2008; 60(1):117–20. [CrossRef]
16. Duarte RF, Sánchez-Ortega I, Cuesta I, Arnan M, Patiño B, Fernández de Sevilla A, et al. Serum galactomannan-based early detection of invasive aspergillosis in hematology patients receiving effective antimold prophylaxis. Clin Infect Dis 2014; 59(12):1696-702. [CrossRef]
17. Metan G, Türe Z, Pala Ç, Kaynar L, Yıldırım A, Elmalı F, et al. A Single Center Experience for Antifungal Prophylaxis in Patients with Acute Myelogenous Leukemia. Indian J Hematol Blood Transfus 2015; 31(3):339-45. [CrossRef]
18. Mennink-Kersten MA, Ruegebrink D, Klont RR, Warris A, Blijlevens NM, Donnelly JP, Verweij PE.. Improved detection of circulating Aspergillus antigen by use of a modified pretreatment procedure. J Clin Microbiol 2008; 46(4): 1391–7. [CrossRef]
19. 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. [CrossRef]
20. Weisser M, Rausch C, Droll A, Simcock M, Sendi P, Steffen I, et al. Galactomannan does not precede major signs on a pulmonary computerized tomographic scan suggestive of Invasive Aspergillosis in patients with hematological malignancies. Clin Infect Dis 2005; 41(8):1143-9. [CrossRef]
21. Herbrecht R, Patterson TF, Slavin MA, Marchetti O, Maertens J, Johnson EM, et al. Application of the 2008 definitions for invasive fungal diseases to the trial comparing voriconazole versus Amphotericin B for therapy of invasive aspergillosis. A collaborative study of the Mycoses Study Group (MSG 05) and the EORTC Infectious Diseases Group. Clin Infect Dis 2015; 60(5):713–20. [CrossRef]
22. Donnelly P, Cordonnier C, Cuenca-Estrella M, Herbrecht R, Kibbler CC, Lass-Flörl CC, et al. A European period-prevalence study to estimate the rate of invasive pulmonary mould disease (PIMDA study). 24th European Congress of Infectious Diseases and Clinical Microbiology, Barcelona, 2014, p0028a.
23. Couchepin J, Brunel AS, Jaton K, Meylan P1, Bochud PY, Lamoth F. Role of bi-weekly serum galactomannan screening for the diagnosis of invasive aspergillosis in haematological cancer patients. Myoses 2018 doi: 10.1111/myc.12755 (Epub a head of print.). [CrossRef]
24. Soyer N, Kiper Ünal HD, Vural F, Şahin F, Töbü M, Dönmez A, et al. Epidemiology and analysis of invasive fungal infections in patients with hematological malignancies: a single-center real-life experience. Turk J Med Sci 2017; 47(5):1535-42. [CrossRef]
25. Avcu G, Karapinar DY, Akinci AB, Sivis ZO, Sahin A, Bal ZS, et al. Utility of the serum galactomannan assay for the diagnosis of invasive aspergillosis in children with acute lymphoblastic leukemia. Int J Infect Dis 2017; 54:8-12. [CrossRef]
26. Sav H, Atalay MA, Koc AN, Unal E, Demir G, Zararsiz G. Utility of the Aspergillus galactomannan antigen testing for neutropenic paediatric patients. Infenz Med 2017; 25(1):38-44.
27. Akan H. Antifungal prophylaxis in stem cell transplantation centers in Turkey. Turk J Haematol 2011; 28:271-5.[CrossRef]