ORIGINAL ARTICLE
Iran J Allergy Asthma Immunol
September 2010; 9(3): 149-155.

The Accuracy of Serum Galactomannan Assay in Diagnosing Invasive Pulmonary Aspergillosis

Shokooh Azam Sarrafzadeh [1], Ali Hoseinpoor Rafati [1], Maryam Ardalan [1], Davood Mansouri [2], Payam Tabarsi [2], and Zahra Pourpak [1]

[1] Immunology, Asthma & Allergy Research Institute, Tehran University of Medical Sciences, Tehran, Iran
[2] Division of Infectious Diseases and Clinical Immunology, National Research Institute & Tuberculosis and Lung Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Received: 1 May 2010; Received in revised form: 25 May 2010; Accepted: 1 June 2010

ABSTRACT

Galactomannan (GM) antigen is an aspergillus specific antigen that is released during the growth phase of invasive aspergillosis. We aimed to find the optimum cut-off and accuracy of serum Galactomannan assay in immunocompromised patients.

Immunocompromised patients diagnosed with invasive pulmonary aspergillosis (IPA) based on the European Organization for Research and Treatment of Cancer/Invasive Mycosis Study Group (EORTC/MSG) with three levels of certainty proven, probable and possible, referred for GM antigen measurement at Immunology, Asthma and Allergy Research Institute (IAARI) from 2006 to 2009 and if they met the criteria were enrolled in this study.

Totally 49 patients with IPA were enrolled in our study. According to EORTC/MSG, patients categorized into three levels of certainty: They were diagnosed as 'proven' invasive pulmonary aspergillosis 16(32.7%), 'probable' 18(36.7%) and 'possible' 15(30.6%). The most common host risk factor was solid tumors 17(34.7%). The accuracy of Galactomannan assay increased from 0.5 to 2 cut-offs. The optimum sensitivity and specificity obtained at the index cut-off of ≥1.5 for diagnosis of "proven" IPA; which were respectively, 69.2% and 72.2%. Other cut-offs had high variance between sensitivity and specificity for diagnosis of IPA.

The calculated cut-off gained by receiver operating characteristic (ROC) analysis for detecting proven IPA was 1.5. Intermediate accuracy of serum GM test in conjunct with clinical findings would help early IPA detection among immunocompromised patients.

Key words: Invasive Pulmonary Aspergillosis; Immunocompromised; Serum Galactomannan

Corresponding Author: Zahra Pourpak, MD, PhD; Immunology, Asthma & Allergy Research Institute, Tehran University of Medical Sciences, Tehran, Iran. Tel: (+98 21) 6691 9587, Fax: (+98 21) 6642 8995. E-mail: pourpakz@sina.tums.ac.ir

INTRODUCTION

Aspergillus spp are ubiquitous and found in nearly everywhere (soil, plant debris and the indoor

environment, especially in hospitals). Clinical manifestations of Aspergillus, commonly A. fumigates in lung are wide spectrum. Immunocompromised patients are prone to life threatening Aspergillus spp infections that are acquired by inhalation of airborne spores.[1] Further more, invasive pulmonary aspergillosis (IPA) is severe disease with its major impact to death among immunocompromised patients. Even critically ill patients without any classic risk factors may develop IPA. Recent advances have led to early diagnosis of IPA by detecting Aspergillus antigens in body fluids. A cell-wall polysaccharide component called galactomannan is detectable during Aspergillus growth. The diagnosis of IPA is simplified by a double-sandwich ELISA which is a FDA (Food and Drug Administration) approved technique for the detection of galactomannan in serum with a threshold of 0.5 ng/ml. One of the superiorities of this technique is its early serum detection of galactomannan prior to presentation of clinical signs, an abnormal chest radiograph, or positive culture. Thus, commencement of earlier treatment and serial serum galactomannan assay is possible for follow-up of patients.[2,3] In this study, we mainly aimed to find the optimum cut-off and accuracy of serum galactomannan assay in immunocompromised patients which have not been previously performed in Iran .

PATIENTS AND METHODS

Totally 49 patients with IPA enrolled with complete clinical and histopathological information. The mean age of patients was 25.3±1.2 (Range: 4-54) years. Most of them were males, 31(63.3%) and male to female ratio was (1.7:1).

Identification of Patients and Definitions:

During 2 years period (from 2006 to 2009), about 49 consecutive immunocompromised patients with the diagnosis of IPA were referred to Immunology, Asthma and Allergy Research Institute, (IAARI).The list of main risk factors were as following bone marrow/solid transplant ,chronic granulomatose diseases (CGD), leukemia and solid tumors. Three levels of certainty (Proven, Probable and Possible) for diagnosis of IPA were defined according to European Organization for the Research and Treatment of Cancer/Invasive Mycosis Study Group (EORTC/MSG) criteria.[4] In each case 2 ml of whole blood was collected by venipuncture, centrifuged and the sera stored in -70 to be used for Galactomannan(GM) Ag test. To make the research in blind method and to prevent any bias, patients were coded and the results of tests were assigned to the codes. The aspergillus GM antigen was detected in serum by direct double-sandwich ELISA (platelia® aspergillus; Bio Rad, Marnes- lacouette, France). The plates were read at an optical density of 450 nm (OD450) with a reference filter of 620/630 nm. An OD index of 0.5 was considered positive. All positive samples considered positive only if the repeated test was also positive.

Mycological Studies:

Sputum or BAL was homogenized and subjected to direct microscopy using 10% KOH and Lactophenol Cotton Blue (LPCB) mount. Fungus culture was performed by plating clinical specimens on to Sabouraud Dexterose Agar and the plates were incubated at 37°C for 3-6 days. Aspergillus species were identified by their culture characteristics and morphologies.[5]

Data Analysis

The optimal cut-off for serum GM Ag testing was determined by receiver operating characteristic (ROC) analysis to discriminate patients with “proven” diagnosis of IPA from patients with “probable or possible” diagnosis. The sensitivity, specificity, PPV, and negative predictive value (NPV) were calculated for serum GM Ag testing according to different cut-offs. The impact of factors associated with IPA were determined with Chi2 test, t test; also the mean of GM Ag calculated in three levels of certainty with ANOVA. P values of <0.05 were considered significant. All analyses performed by SPSS 16 for windows.

RESULTS

Clinical presentations of the patients included cough 49(100%), sputum 46(93.9%), fever 43(87.8%). The mean duration of symptoms was 2.8±2.1 (range: 1-10) months. Lung CT findings favoring invasive aspergillus reported in 43(87.8%) patients. The most common underling host risk factor was solid tumors 17(34.7%) followed by bone marrow/ solid transplant 15(30.6%), chronic granulomatous disease 9(18.4%), and leukemia 8(16.3%). The most common type of Aspergillus was Fumigatus 44(89.7%) and other less

frequent types were Flavous and Niger, respectively 3 (6.1%) and 2(4.1%).

Patients' characteristics according to two levels of certainty ("proven" versus "probable or possible") have been summarized in table 1. Bivariate analysis showed that gender, fever, sputum, bronchiectasia, lung CT findings, associated diseases and aspergillus type had no relationship with the diagnosis of IPA. (p>0.05) (Table 1). Also, age and duration of symptoms did not have significant impact on IPA diagnosis (p>0.05).

Regarding (EORTC/MSG), the number of patients in each group were as followed 'proven' 16(32.7%), 'probable' 18(36.7%) and 'possible' 15(30.6%). The mean of Galactomannan(GM) Ag in these groups consisted of: 'Proven': GM 1.79 ± 0.87; 'Probable': GM 1.04 ± 0.55; 'Possible': GM 0.98 ± 0.51.The mean of Galactomannan Ag was significantly different among three levels of certainty (ANOVA, p=0.002)

The sensitivity, specificity, PPV, and NPV of serum GM testing at various interpretive cut-offs are summarized in Table 2. A cut-off of _0.5 yielded sensitivity and NPV of 100%, with relatively low specificity and PPV. The specificity and accuracy improved by increasing the cut-off from 0.5 to 2, as shown in the ROC curve (Figure 1). The area under curve (AUC) found to be statistically significant, (AUC: 0.812(CI 95%: 0.676_ 0.947), p=0.0001)

Table 1. The relationships between different variables and IPA diagnosis

Variables		Diagnostic groups		P-value
		Proven	Probable or possible	
Gender	Male	12(75%)	19(57.6%)	0.23
	Female	4(25%)	14(42.4%)	
Sputum	Positive	12(92.3%)	33(97.1%)	0.47
Fever	Positive	12(92.3%)	29(85.3%)	0.51
Bronchiectasia	Positive	12(92.3%)	33(97.1%)	0.47
Lung CT findings	Positive	13(100%)	30(88.2%)	0.19
	Fumigatus	12(92.3%)	32(88.8%)	0.33
Aspergillus Type	Flavus	0 (.0%)	3(8.3%)	
	Niger	1(7.7%)	1(2.8%)	
	CGD	5(38.5%)	4(11.1%)	0.27
Host risk factors	*BM/ST	4(30.8%)	11(30.6%)	
	Solid tumor	3(23%)	14(38.9%)	
	Leukemia	1(7.7%)	7(19.4%)	

*BM/ST: Bone marrow/ solid transplant

Table 2. The accuracy of serum GM test with different cut-off points

Serum GM cut-offs	Sensitivity	Specificity	PPV	NPV	Accuracy
≥0.5	100(13/13)	22.2(8/36)	31.7(13/41)	100(8/8)	43(21/49)
≥1	77(10/13)	39(14/36)	31.2(10/32)	82.4(14/17)	49(24/49)
≥1.5	69.2(9/13)	72.2(26/36)	47.4(9/19)	86.7(26/30)	71.4(35/49)
≥2	30.8(4/13)	97.2(35/36)	80(4/5)	79.5(35/44)	79.5(39/49)

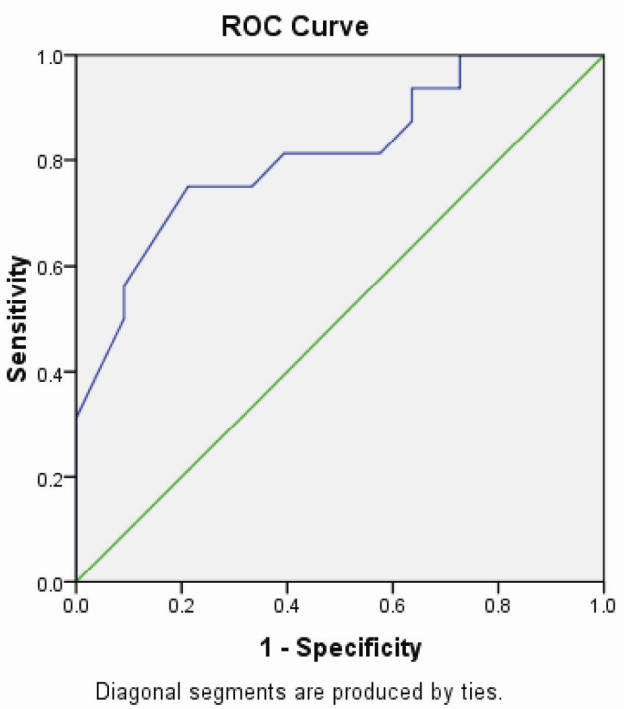

Figure 1. The ROC Curve of Galactomannan antigen (EIA) test for diagnosing patients with proven IPA

DISCUSSION

IPA is diagnosed definitely by invasive techniques such as biopsy for histopathology assay.[4] However; conventional culture from BAL fluid samples may rise to a relatively low sensitivity for detection of A. fumigatus in patients with IPA.[6-8]

Thus , more sensitive and specific diagnostic tests with the least invasive nature are required , such as GM EIA which would help clinicians to initiate antifungal therapy in the absence of obvious clinical symptoms in patients with a high probability of IPA, such as in high risk populations with neutropenia and malignancy or patients that have undergone transplantation.

In literature review, numerous studies[9-23] provided variable sensitivity and specificity of the GM Ag assay in different patient populations. In the present study, we considered a positive galactomannan test result for defining proven cases in according to EORTC/MSG criteria. The ROC revealed that the galactomannan Ag test for serum indicated 69% sensitivity and 70% specificity at an optimal GMI cut-off of 1.5. However, determining the performance of the test separately by regarding different host risk factors was not possible, because of our small sample size. A decade ago, a cut-off serum ratio of 1.5 was recommended in the manufacturers' manual in Europe .However, currently many studies have used a cut-off value of 1.0, and the US Food and Drug Administration in the United States recommended a cut-off value of 0.5. Actually, we faced improved accuracy of the test with higher thresholds, and the point of 1.5 was considered optimal (Table A). Our findings with some tolerance are in agreement with the comprehensive meta-analysis study performed by Pfeiffer et al.[24] They reviewed twenty-seven studies from 1996 to 2005 to determine the accuracy of galactomannan test for diagnosing IPA. As a whole, the serum GM test had a sensitivity of 71% and specificity of 89% for proven cases of invasive Aspergillosis. The negative predictive value was 92–98% and the positive predictive value was 25–62%. The comparative review of different studies has been shown in table. A. This variance in the results of mentioned studies may be attributed in part to medications such as b-lactam antibiotics which is associated with a false-positive assay, or antifungal agents against Aspergillus which may lead to false-negative results.[5-27]

Table A. The comparison of GM EIA tests accuracy in different studies

Study	year	Threshold	Sensitivity	Specificity
Verweij et al. (9)	1995	1	.83	.65
Sulahian et al. (10)	1996	1	.76	.72
Bretagne et al. (11)	1997	1	1	.72
Bretagne et al. (12)	1998	1	1	.63
Machetti et al. (13)	1998	1.5	1	.76
Ulusukarya et al. (15)	2000	1	.8	.93
Becker et al. (6)	2003	1	0.50	0.80
Suhalian et al. (16)	2001	1.5	.77	.93
Kami et al. (17)	2001	1.5	.58	.97
Costa et al. (18)	2002	1.5	1	.38
Herbrecht et al. (5)	2002	1.5	.65	.92
Maertens et al. (19)	2002	1	1	.86
Moragues et al. (14)	2003	1.5	.67	.98
Pazos et al. (20)	2003	1.5	1	.97
Challier et al. (21)	2004	1	.67	.77
Marr et al. (3)	2004	1	.62	.7
Rovira et al. (22)	2004	1.5	1	.93
Yoo et al. (23)	2005	.5	.5	.71
This study	2010	1.5	.69	.72

Finally, as galactomannan test is not species-specific such as Fumigatus and it does not rule out the possibility of other types such as Fusarium, Zygomycetes, and diatomaceous fungi,[28] detailed clinical and histopathologic evaluations are still essential.

CONCLUSION

The findings of our study were in line with other similar studies. The calculated cut-off gained by ROC for detecting IPA was 1.5. According to the present study, because of intermediate accuracy of serum galactomannan test for diagnosis of IPA in immunocompromised patients, this test in conjunct with clinical findings would serve for early IPA detection.

REFERENCES

1. Soubani AO, Chandrasekar PH. The clinical spectrum of pulmonary aspergillosis. Chest 2002; 121(6):1988-99.
2. Boutboul F, Alberti C, Leblanc T, Sulahian A, Gluckman E, Derouin F, et al. Invasive aspergillosis in allogeneic stem cell transplant recipients: increasing antigenemia is associated with progressive disease. Clin Infect Dis 2002; 34(7):939-43.
3. Marr KA, Balajee SA, McLaughlin L, Tabouret M, Bentsen C, Walsh TJ. Detection of galactomannan antigenemia by enzyme immunoassay for the diagnosis of invasive aspergillosis: variables that affect performance. J Infect Dis 2004; 190(3):641-9.
4. Ascioglu S, Rex JH, de Pauw B, Bennett JE, Bille J, Crokaert F, et al. 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.
5. Herbrecht R, Letscher-Bru V, Oprea C, Lioure B, Waller J, Campos F, et al. Aspergillus galactomannan detection in the diagnosis of invasive aspergillosis in cancer patients. J Clin Oncol 2002; 20(7):1898-906.
6. Becker MJ, de Marie S, Willemse D, Verbrugh HA, Bakker-Woudenberg IA. Quantitative galactomannan detection is superior to PCR in diagnosing and monitoring invasive pulmonary aspergillosis in an

experimental rat model. J Clin Microbiol 2000; 38(4):1434-8.
7. Levy H, Horak DA, Tegtmeier BR, Yokota SB, Forman SJ. The value of bronchoalveolar lavage and bronchial washings in the diagnosis of invasive pulmonary aspergillosis. Respir Med 1992; 86(3):243-8.
8. Reichenberger F, Habicht J, Matt P, Frei R, Soler M, Bolliger CT, et al. Diagnostic yield of bronchoscopy in histologically proven invasive pulmonary aspergillosis. Bone Marrow Transplant 1999; 24(11):1195-9.
9. Verweij PE, Stynen D, Rijs AJ, de Pauw BE, Hoogkamp-Korstanje JA, Meis JF. Sandwich enzyme-linked immunosorbent assay compared with Pastorex latex agglutination test for diagnosing invasive aspergillosis in immunocompromised patients. J Clin Microbiol1995; 33(7):1912-4.
10. Sulahian A, Tabouret M, Ribaud P, Sarfati J, Gluckman E, Latge JP, et al. Comparison of an enzyme immunoassay and latex agglutination test for detection of galactomannan in the diagnosis of invasive aspergillosis. Eur J Clin Microbiol Infect Dis 1996; 15(2):139-45.
11. Bretagne S, Marmorat-Khuong A, Kuentz M, Latge JP, Bart-Delabesse E, Cordonnier C. Serum Aspergillus galactomannan antigen testing by sandwich ELISA: practical use in neutropenic patients. J Infect 1997; 35(1):7-15.
12. Bretagne S, Costa JM, Bart-Delabesse E, Dhedin N, Rieux C, Cordonnier C. Comparison of serum galactomannan antigen detection and competitive polymerase chain reaction for diagnosing invasive aspergillosis. Clin Infect Dis 1998; 26(6):1407-12.
13. Machetti M, Feasi M, Mordini N, Van Lint MT, Bacigalupo A, Latge JP, et al. Comparison of an enzyme immunoassay and a latex agglutination system for the diagnosis of invasive aspergillosis in bone marrow transplant recipients. Bone Marrow Transplant 1998; 21(9):917-21.
14. Moragues MD, Amutio E, Garcia-Ruiz JC, Ponton J. Usefulness of galactomannan detection in the diagnosis and follow-up of hematological patients with invasive aspergillosis. Rev Iberoam Micol 2003; 20(3):103-10.
15. Ulusakarya A, Chachaty E, Vantelon JM, Youssef A, Tancrede C, Pico JL, et al. Surveillance of Aspergillus galactomannan antigenemia for invasive aspergillosis by enzyme-linked immunosorbent assay in neutropenic patients treated for hematological malignancies. Hematol J 2000; 1(2):111-6.
16. Sulahian A, Boutboul F, Ribaud P, Leblanc T, Lacroix C, Derouin F. Value of antigen detection using an enzyme immunoassay in the diagnosis and prediction of invasive aspergillosis in two adult and pediatric hematology units during a 4-year prospective study. Cancer 2001; 91(2):311-8.
17. Kami M, Fukui T, Ogawa S, Kazuyama Y, Machida U, Tanaka Y, et al. Use of real-time PCR on blood samples for diagnosis of invasive aspergillosis. Clin Infect Dis 2001; 33(9):1504-12.
18. Costa C, Costa JM, Desterke C, Botterel F, Cordonnier C, Bretagne S. Real-time PCR coupled with automated DNA extraction and detection of galactomannan antigen in serum by enzyme-linked immunosorbent assay for diagnosis of invasive aspergillosis. J Clin Microbiol 2002; 40(6):2224-7.
19. Maertens J, Van Eldere J, Verhaegen J, Verbeken E, Verschakelen J, Boogaerts M. Use of circulating galactomannan screening for early diagnosis of invasive aspergillosis in allogeneic stem cell transplant recipients. J Infect Dis 2002; 186(9):1297-306.
20. Pazos C, del Palacio A. Early diagnosis of invasive aspergillosis in neutropenic patients with bi-weekly serial screening of circulating galactomannan by Platelia Aspergillus. Rev Iberoam Micol 2003; 20(3):99-102.
21. Challier S, Boyer S, Abachin E, Berche P. Development of a serum-based Taqman real-time PCR assay for diagnosis of invasive aspergillosis. J Clin Microbiol 2004; 42(2):844-6.
22. Rovira M, Jimenez M, De La Bellacasa JP, Mensa J, Rafel M, Ortega M, et al. Detection of Aspergillus galactomannan by enzyme immunoabsorbent assay in recipients of allogeneic hematopoietic stem cell transplantation: a prospective study. Transplantation 2004; 77(8):1260-4.
23. Yoo JH, Choi JH, Choi SM, Lee DG, Shin WS, Min WS, et al. Application of nucleic acid sequence-based amplification for diagnosis of and monitoring the clinical course of invasive aspergillosis in patients with hematologic diseases. Clin Infect Dis 2005; 40(3):392-8.
24. Pfeiffer CD, Fine JP, Safdar N. Diagnosis of invasive aspergillosis using a galactomannan assay: a meta-analysis. Clin Infect Dis 2006; 42(10):1417-27.
25. Singh N, Obman A, Husain S, Aspinall S, Mietzner S, Stout JE. Reactivity of platelia Aspergillus galactomannan antigen with piperacillin-tazobactam: clinical implications based on achievable concentrations in serum. Antimicrob Agents Chemother2004; 48(6):1989-92.

26. Marr KA, Laverdiere M, Gugel A, Leisenring W. Antifungal therapy decreases sensitivity of the Aspergillus galactomannan enzyme immunoassay. Clin Infect Dis 2005; 40(12):1762-9.
27. Ansorg R, van den Boom R, Rath PM. Detection of Aspergillus galactomannan antigen in foods and antibiotics. Mycoses 1997; 40(9-10):353-7.
28. Busca A, Locatelli F, Barbui A, Limerutti G, Serra R, Libertucci D, et al. Usefulness of sequential Aspergillus galactomannan antigen detection combined with early radiologic evaluation for diagnosis of invasive pulmonary aspergillosis in patients undergoing allogeneic stem cell transplantation. Transplant Proc 2006; 38(5):1610-3.