

Evaluation of Two Readings for the QuicGM *Aspergillus* Lateral Flow Assay in a Group of Immunocompromised Patients in Iraq

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Abstract

Background: *Aspergillus* spp. causes a wide range of diseases called Invasive Aspergillosis (IA) which is a fatal illness that affects a variety of immunocompromised people worldwide.

Objective: Using the lateral flow test for the detection of Galactomannan antigen in immunocompromised patients suspected to have IA.

Patients and Methods: This study was conducted on 72 patients, whose samples (serum, Bronchoalveolar lavage, Blood) were collected from the Hematology-Oncology Unit at Baghdad Teaching Hospital and Pediatric Welfare Hospital, and ICU in Ghazi AL-Hariri Surgical Specialties Hospital, and Bone Marrow Transplantation Center in period between November 2022 to February 2023. Patients' blood and sputum were sent for fungal culture to confirm the diagnosis in most cases.

Results: QuicGM *Aspergillus* Lateral Flow Assay was conducted on 72 patients, of whom 34 (47.2%) were positive and 38 (52.7%) were negative (*P*-value of 0.001 and 0.5) respectively. This screening aimed at detecting IA. One week later, the second confirmative result was obtained from 24 patients to determine the response to antifungal drugs or recovery from neutropenia on which 15 readings were negative and nine were positive. Out of 48 single readings, 25 were positive and 23 were negative. All for 72 members of the control group gave negative results. This study is the first in Iraq to use this assay.

Conclusion: QuicGM *Aspergillus* Lateral Flow Assay was found to be reliable, sensitive, and specific, and proved to be a very good guide for the early diagnosis of IA in immunocompromised patients and in monitoring treatment outcomes and follow-up.

Keywords: *Aspergillus* diagnostics; Galactomannan; Invasive Aspergillosis (IA); Lateral Flow Assay.

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Introduction:

Invasive Aspergillosis (IA) is a fatal fungal illness that affects a variety of immunocompromised persons worldwide, including people with immunocompetent and non-neutropenic lung disease and viral or bacterial pulmonary infections, as well as those with hematological malignancies receiving chemotherapy and/ or immunosuppressive/ immunomodulatory drugs, neutropenia, stem cell transplantation, and organ transplantation (1). IA is caused by the very common and opportunistic fungus called *Aspergillus* spp (2). *Aspergillus* spp. is the second most common cause of fungus-related respiratory infections in critically ill patients, following fungi from the order Mucorales. They are not a part of the normal flora. There are numerous species of *Aspergillus* that are common in the environment around us, including *Aspergillus flavus* which represents the more common type of fungus that can grow on fruits, vegetables, and even in the air and soil(3).

Regular inhalation of their spores has no adverse side effects, but some species, most notably *A. fumigatus*, are capable of spreading a variety of diseases, including allergic bronchopulmonary Aspergilloma, when the fungus spreads from the lungs, and causes widespread illness in the immunocompromised patient (4). Morphological and microscopic methods were used to identify this mold. The identification process was based on cultural characteristics such as colony morphology, the presence of septate hyphae, and the shape of conidial heads (5). The Taiwan Food and Drug Administration (TFDA) approved the Galactomannan (GM) assay in 2003 as a widely used enzyme immunoassay for the detection of IA. Galactomannan, a polysaccharide component of the *Aspergillus* cell wall produced in varying amounts by the fungus hyphae in the serum and nearby fluids of infected organs such as bronchoalveolar lavage (BAL) during invasive growth (6). It had been discovered by Reiss and Lehman as a potential indicator IA (7). For the diagnosis of the more invasive form of aspergillosis. The detection of GM might be regarded as a suitable assay.

The benefit of utilizing this assay, particularly its capacity to identify IA in its earliest stages (8). However, the GM assay has several methodological limitations since the test findings can be influenced

by many circumstances and because it takes a lot of time and labor (9). The early diagnosis of IA is particularly important, the BAL fluid/serum Galactomannan assay is a useful auxiliary diagnostic modality. Since the sensitivity of existing microbiological procedures is low, they take a long time to complete. Lateral flow assays (LFA) for the diagnosis of IA have recently been CE (Conformité Européenne) marked and commercialized. These assays can be used to test samples quickly (10), (11). Galactomannan cannot be used as a diagnostic indicator by itself. Clinical correlation, radiologic results, and underlying risk factors are all important considerations when deciding whether to start empiric treatment (12). It was done by using QuicGM *Aspergillus* LFA is based on fluorescent immunochromatography (13), (14). The main goal of our research is to aid physicians in rapid assessment of patients with invasive aspergillosis and monitoring patients during course of treatment with antifungal drugs. Patients who benefit from it is hematological malignancies with neutropenia, allogeneic hematopoietic stem cell transplantation, glucocorticoids use for over three weeks, immunosuppressant use, graft-versus-host disease. This assay may evolve to include all body fluids such as urine and cerebrospinal fluid.

Patients and Methods:

Seventy-two patients were included in this study. Samples of serum and Bronchoalveolar lavage were collected from patients in the Hematology-Oncology Unit at Baghdad Teaching Hospital and Pediatric Welfare Hospital (52 patients), Bone Marrow Transplantation Center (six patients), intensive care unit (ICU) in Ghazi Al-Hariri Surgical Specialties Hospital (four patients), and respiratory care unit (RCU) (10 patients). All of these were referred by Hematology-Oncology department specialists. Blood and sputum samples were cultured to confirm diagnosis. Seventy-two healthy controls were also enrolled in this study, selected as a disease-free and immunocompetent and healthy people from the community, their age ranged from 14-64 years old, while the patients' age ranged from 1-80 years. The data for the study was collected between November 2022 to February 2023. Azole drugs included in the present study are fluconazole and voriconazole. Mixed include combination of voriconazole + traditional amphotericin, caspofungin + liposomal amphotericin, fluconazole+ liposomal amphotericin. Other antifungal drugs used for suspected IA with non-diagnostic causes. Point of care testing was performed using the QuicGM LFT according to the manufacturer's guidelines. Briefly, 300 μ L of serum/BAL pipetted into 1.5 ml screw-cap polypropylene tubes. 100 μ L of sample treatment solution added to the tubes containing the serum/BAL, then vortexed for 10 seconds to thoroughly mix the contents. The tubes were centrifuged at 10,000 \times g for 1-5 seconds to shake the sample out of the tubes. The tube was heated in a

water bath for 3-4 minutes at 100 °C. The tubes were centrifuged for 10 minutes at 10,000 g, then the supernatant was collected for testing. This assay was conducted on different groups patients within the Medical City Hospitals suspected of having invasive fungal infections, serving as a rapid means that take around (45 minutes) of identifying infection before initiating treatment thereby avoiding empirical therapy. Subsequently, in cases where the LFT yielded a positive outcome, coupled with a positive culture and corresponding positive radiological findings, the researchers administered tailored treatment based on medical assessment. Most of the patients were suffering from potential fungal infections affecting the lungs as observed through MRI or CT, complemented by sputum samples and blood culture.

Statistical Analysis:

All data of the current study were analyzed by using Statistical Package for Social Science version 26 and Excel 2016. Scale parameters were calculated by student t-test, and P values were less than 0.05 were considered significant. Sensitivity and specificity were calculated by receiver operating characteristic curve (ROC curve).

Results:

Of the 72 cases, 31 (43.1%) were females and 41 (56.9%) were males. The patients age range was 1-80 years. The control group consisted of 72 healthy persons who were negative for IA. Out of 72 blood cultures among the cases, 27 cases were positive for the following organisms (*Candida* spp. 10, *Aspergillus* spp. 9, *Cryptococcus* 8, Gram negative bacteria 5, *Sporothrix schenckii* 1, *Histoplasma capsulatum* 1). For sputum culture, only 15 out of 31 were positive for the following organisms *Candida* spp. 13, *Cryptococcus* 4, Gram-negative bacteria 2, *Aspergillus* spp. as shown in figure (1).

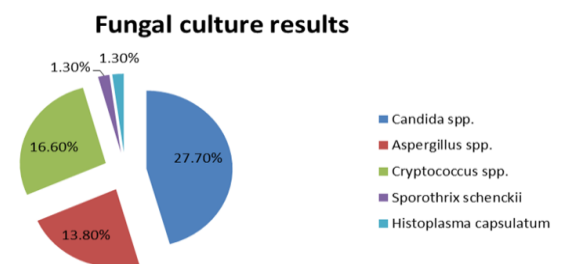


Figure (1): Distribution of types of microorganisms from blood and sputum cultures.

In this study, 72 LFT were conducted on the samples of 72 patients: 48 patients were with a single reading, of whom 25 (52.1%) were positive and 23 (47.9%) were negative (P-value 0.079 and 0.002, respectively, calculated by t-test) this is not a construction, t-test used in this study to see the significant differences between the means of two Galactomannan result, the percentage was demonstrated to show number of positive cases to be negative once. Most patients in

this study were neutropenic (61%) (500-1000 cell/ml). A second reading was taken after one week for 24 patients to determine the response to antifungal drug or recovery from neutropenia, 9 of whom (37.5%) were positive and 15 (62.5%) were negative (P-value of 0.01 and 0.012, respectively, calculated by t-test) table 1. There was no significant difference between the first and the second readings of QuicGM *Aspergillus* LFT. Thirty-four (47.2%) tests were positive and 38 (52.7%) were negative (P-value of 0.000 and 0.506) respectively, calculated by t-test)

Table 1: The mean values for the QuicGM LFT first and second readings

LFT reading	No.	Mean	±SD	P value(t-test)
Single				
+ve	25	1.17	1.055	0.079
-ve	23	0.35	0.099	0.002
Total	48	0.76	0.850	0.488
Second				
+ve	9	0.74	0.208	0.01
-ve	15	0.34	0.100	0.012
Total	24	0.49	0.245	0.288

The current study found that there is a highly significant difference between patients who had a positive fungal growth in either blood or sputum culture where 16 patients showed a positive LFT and 20 patients showed a negative LFT, (P-value < 0.001 calculated by t-test) table 2.

Table (2): Relationship between positive fungal growth and the readings of QuicGM *Aspergillus* LFT

Fungal growth	LFT Level	N	Mean	Std. Deviation	P – Value
Positive growth	+	16	0.91	0.318	< 0.001
	-	20	0.32	0.101	
	Total	36			

According to antifungal treatment, for patients treated with traditional Ambisome, four of them were positive for LFT, while seven patients were negative, (P -value < 0.003, calculated by t-test). Twenty patients were treated with liposomal amphotericin B, of whom only nine were positive for LFT and 11 were negative with highly significant differences (P-value 0.001, calculated by t-test). Nine patients were treated with caspofungin, seven of whom were positive and two were negative for the LFT with a highly significant difference (P-value 0.001, calculated by t-test). Other azole drugs were used (fluconazole and voriconazole) for three LFT positive cases and seven negative patients, (P-value 0.015, calculated by t-test). Drug combinations include (voriconazole + traditional ambisome), (caspofungin + liposomal amphotericin), (fluconazole + liposomal amphotericin), for five positive and five negative patients (P-value 0.008, calculated by t-test). Other antifungals drugs were used for suspected IA and non-diagnostic causes for five positive and five negative patients (P-value 0.014, calculated by t-test), table (3).

Table 3: Relationship between the type of antifungal drugs used and results of QuicGM *Aspergillus* LFT

Antifungal	LFT Level	N	Mean	Std. Deviation	P – Value
Traditional AB	+	4	2.145	2.503	0.003
	-	7	0.37	0.084	
	Total	11			
Liposomal	+	9	1.03	0.406	0.001
	-	11	0.39	0.091	
	Total	20			
Caspofungin	+	7	0.89	0.270	0.001
	-	2	0.24	0.049	
	Total	9			
Azole	+	3	0.73	0.272	0.015
	-	7	0.32	0.158	
	Total	10			
Mixed	+	5	0.79	0.262	0.008
	-	5	0.334	0.109	
	Total	10			
Other	+	5	0.91	0.142	0.014
	-	5	0.29	0.142	
	Total	10			

Forty-four out of seventy-two patients had neutropenia 44 (61.1%), 19(43.1%) of them were positive for QuicGM *Aspergillus* LFT and 25 (56.8%) of them were negative for QuicGM *Aspergillus* LFT with significant difference P-Value (<0.05) as in table (4).

Table 4: Relationship of neutropenia with QuicGM *Aspergillus* LFT readings :

	LFT Level	N	Mean	Std. Deviation	P - Value
Low WBC (neutropenia)	+	19	0.87	0.344	< 0.05
	-	25	0.384	0.260	
	Total	44			

Standard curve:

QuicGM *Aspergillus* LFA (using 0.5 cutoffs) had a sensitivity of 89% and a specificity of 100%. Figure (2)

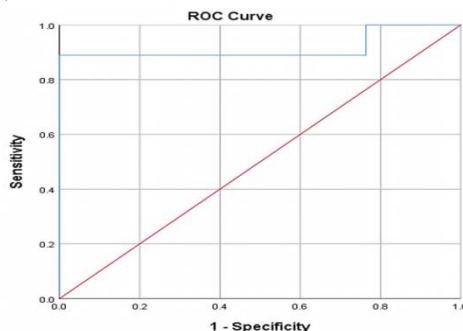


Figure (2): The standard curve

Discussion:

QuicGM *Aspergillus* Lateral Flow Assay is one of the novel methods for IA diagnosis. (15). The LFA is a self-contained fluorescent immunochromatographic assay for the qualitative and quantitative identification of *Aspergillus* GM in serum and BAL sample. (16) QuicGM *Aspergillus* LFT was widely used in the hospitals of Belgium, a wide range of LFAs are implemented in Jodhpur, Rajasthan, and Turkey. (17) (18) (19) The LFT test was conducted twice on 24 patients, initially on days

1 and 7 days later, with the majority of the patients having decreased Galactomannan levels in the second test, as a result of their positive response to the treatment they received. Follow up of patients is very useful in two readings measure within the first week of antifungal therapy as rising titer refers to active fungal infections while decreased values refereed to good response to adequate antifungal therapy. In the current study, the second test revealed lower Galactomannan antigen levels in seven patients, and that was in agreement with Taghavi et al. who stated that patients with a high risk of IA should have a baseline serum test and have their levels of GM antigen monitored twice a week. (20)

The distribution of the study groups based on blood disease data showed that IA is more prevalent in Acute lymphoblastic leukemia (ALL) due to lack of lymphocyte generation and trafficking, as well as changes in the way lymph organs operate, which are characteristics of lymphocytic leukemia. Contrary to earlier research that claimed patients with acute myeloid leukemia most usually develop IA, as the adaptive immune system is linked to modified and defective lymphocytic function. (21) (22) The distribution of fungal infection in blood and sputum samples varied significantly, with the majority of these samples showing yeasts, molds, and Gram-negative bacteria this was in agreement with other studies where the presence of bacteria in the blood is frequently linked to serious diseases. (23) (24) Liposomal amphotericin was the drug of choice for treating invasive pulmonary Aspergillosis (IPA) because it is the medication that is most readily available in Iraq and this was in agreement with other studies. (25) (26) According to the blood count data, the majority of the patients had neutropenia, which is the body's main defense against infection. When exposed to *Aspergillus* spores, neutropenic individuals are more likely to develop a fungal infection, particularly when intense chemotherapy causes the polymorph nuclear cell level to be below 1000 cells/ml. (27)

Conclusion:

QuicGM *Aspergillus* Lateral flow assay can be used as a diagnostic method in conjunction with other diagnostic procedures and as an aid in the diagnosis of IA. The two readings were very useful in the follow up of patients' response to antifungal therapy.

Authors' declaration:

Conflicts of Interest: The authors declare no conflict of interest. We confirm that all the Figures and Tables in the manuscript belong to the current study. Besides, the figures and images, which do not belong to the current study, have been given permission to be republished and attached to the manuscript. Authors sign on ethical consideration's approval-Ethical Clearance: The project was approved by the local ethical committee in (Hematology-Oncology Unit at Baghdad Teaching Hospital and Pediatric Welfare Hospital, and ICU in Ghazi AL-Hariri Surgical Specialties Hospital, and Bone Marrow

Transplantation Center) according to the code number (0211) on (16/ 07/ 2022). Funding: non Limitations: No. of samples

Author contributions: Study conception & design: Wifaq M. AL-Wattar Literature search: Hiba S. Kareem Data acquisition: Hiba S. Kareem Data analysis & interpretation: Hiba S. Kareem. Manuscript preparation: Hiba S. Kareem. Manuscript editing & review: Hiba S. Kareem & Wifaq M. AL-Wattar

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تقييم قراءتين لأختبار التدفق الجانبي السريع لكالاكتومانان الرشاشيات في مجموعة المرضى الذين يعانون من النقص المناعي في العراق

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الخلاصة

الخلفية: يسبب جنس الرشاشيات مجموعة واسعة من مرض داء الرشاشيات والذي هو مرض قاتل يصيب مجموعة متنوعة من الاشخاص الذين يعانون من نقص المناعة في جميع انحاء العالم. يعد الكالاكتومانان علامة حيوية مهمة تم استخدامها تاريخيا في تشخيص ومراقبة الالتهابات الفطرية وخاصة داء الرشاشيات الغازي الذي يمكن اكتشافه في مصل الدم وسائل غسيل القصبات.

الاهداف: استخدام مقياس التدفق الجانبي للكشف عن مستضد الكالاكتومانان في مرضى العوز المناعي الذين يعانون من عدوى فطرية هوائية مشتبه بها.

المرضى والطرائق: اجريت هذه الدراسة على 72 مريضا وتم جمع عيناتهم (مصل الدم، غسيل القصبات) من وحدة امراض الدم والاورام في مستشفى بغداد التعليمي، مستشفى الطفل المركزي، مركز زرع نخاع العظم، ووحدة العناية المركزة في مستشفى غازي الحريري للتخصصات الجراحية بين تشرين الثاني 2022 وشباط 2023. تم إرسال دم المرضى والبلغم لإجراء زراعة فطرية لتأكيد التشخيص في معظم الحالات. باستخدام اختبار التدفق الجانبي السريع للرشاشيات ووسط زرع الدم المناسب ومن ثم زرع النخامة.

النتائج: تم إجراء مقياس التدفق الجانبي للرشاشيات على 72 عينة في قراءة اولى حيث تبين ان 34 (47.2%) كانت موجبة و 38 (52.7%) عينة سالبة وكانت (P-value of 0.001 and 0.5) يهدف هذا الفحص إلى الكشف عن داء الرشاشيات الغازي وبعد أسبوع واحد تم الحصول على النتيجة التأكيدية الثانية من 24 مريضاً لتحديد الاستجابة للأدوية المضادة للفطريات أو التعافي من قلة العدلات حيث كانت (15 كانت سلبية 9 كانت ايجابية) وومن بين 48 قراءة منفردة (25 كانت موجبة و 23 كانت سالبة). كل القراءات ل 72 حاله ضابطة اعطت نتائج سلبية. تعتبر هذه الدراسة الاولى التي استخدمت هذا الاختبار في العراق.

الاستنتاجات: كان مقياس التدفق الجانبي للرشاشيات موثوقاً، ذو حساسية و خصوصية، واثبت انه دليلا جيدا جدا لتشخيص داء الرشاشيات الغازي في مجموعة المرضى الذين يعانون من نقص المناعة ومراقبة متابعة العلاج.

الكلمات المفتاحية: اختبار التدفق الجانبي; الكالاكتومانان; داء الرشاشيات الغازي; تشخيص الرشاشيات