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Original Article

Assessment of Platelia *Aspergillus* enzyme immunoassay for the diagnosis of invasive aspergillosis

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Background and Purpose: This study investigated the diagnostic value of Platelia *Aspergillus* enzyme immunoassay (EIA) for galactomannan (GM) antigen in patients at risk of invasive aspergillosis (IA), and its association with clinical course and outcome.

Methods: A total of 304 blood samples were collected from 189 patients at risk of IA during a 1-year period at a tertiary referral center. Classification of IA was made on the basis of the European Organization for Research and Treatment of Cancer case definitions.

Results: Of the 189 patients, 5 had proven IA, 9 had probable IA, 26 had possible IA, and 149 had no IA. Diagnostic levels of GM were detected in 80% of proven and in 77% of probable IA cases. The overall sensitivity, specificity, and positive and negative predictive values for this assay, using a 1.5 index cut-off value, were 78.6%, 93.9%, 55.0%, and 97.9%, respectively. With the 0.5 index cut-off value, the sensitivity would increase to 100%. A close relationship was found between clinical course and the kinetics of GM indices in survivors.

Conclusions: The Platelia *Aspergillus* EIA is a useful screening test for the detection of IA. Regular monitoring of the kinetics of GM-EIA indices is a useful predictor of clinical course and outcome.

Key words: Aspergillosis; *Aspergillus*; Early diagnosis

Introduction

The incidence of invasive aspergillosis (IA) has significantly increased in neutropenic patients, patients with acquired immunodeficiency syndrome, matched unrelated donor transplant recipients, and patients on aggressive immunosuppressive regimens that impair macrophage function (steroids, chemotherapy, etc.) [1-3]. This severe opportunistic fungal infection is characterized by a high mortality rate in these at-risk patients [4]. Early and accurate diagnosis is very important for instituting an antifungal therapy and improving prognosis.

However, IA is always difficult to diagnose. The diagnosis of IA is based ideally on histological documentation of typical hyphae and a positive culture for *Aspergillus*. In clinical practice, however, obtaining specimens by invasive procedures for such a diagnosis is often impractical due to the clinical condition of these patients, and clinical management must therefore proceed pragmatically by symptomatic, radiological, and microbiological criteria that provide different levels of certainty of the diagnosis. Waiting for definite proof of a diagnosis of IA before therapy is initiated places patients in mortal danger from disease progression. Therefore, use of methods, other than culture, for diagnosing IA is essential.

Several laboratory methods that involve the detection of antigens, metabolites, or nucleic acid have been devised [5]. To detect circulating *Aspergillus* galactomannan (GM), the commercially available enzyme immunoassay (EIA) test (Platelia *Aspergillus* EIA test) is now commonly used as an aid in the diagnosis of IA, and is far more sensitive than the latex

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agglutination test with the same monoclonal antibody [6,7]. However, high variations in sensitivity and specificity have been reported that were mainly related to the patient populations examined, the cut-off value used to define a positive result, and the frequency of the serological survey [6-8]. This study evaluated the utility of Platelia *Aspergillus* EIA test for diagnosing IA in a serological survey of at-risk patients and its correlation with clinical course and outcome.

Methods

Patients and setting

We performed this study from August 2004 to August 2005 at the National Taiwan University Hospital, a tertiary referral center with 1800 beds. Most of the patients in this series, especially those needing critical care, chemotherapy, or transplant, were referred from local hospitals. There were 175 beds in the intensive care unit and 150 beds in the hematology-oncology ward of this hospital during the study period. The following data were collected for each patient: age and gender; predisposing factors for IA, including underlying diseases and associated medical conditions; peripheral white blood cell count and differential cell counts; radiographic findings; strains of pathogens isolated in biopsy specimens; antifungal therapy regimens and durations; invasive or surgical procedures; hospitalization duration; and outcome.

Definition of IA

IA was classified on the basis of the European Organization for Research and Treatment of Cancer/Invasive Fungal Infection Cooperative Group and the National Institute of Allergy and Infectious Disease Mycoses Study Group case definitions [1]. Proven IA diagnosis was based on *Aspergillus* isolation in deep tissues. For a case of IA to be considered "probable", each of the 3 elements — host factor, clinical features, and mycological evidence — has to be present. In contrast, a patient who is positive for at least 1 criterion from the host factors category but does not have clinical features or mycological evidence has a case that can be classified only as "possible." In this study, GM results were excluded as microbiological criteria in these definitions.

Mycological studies

For isolation of fungi, specimens were inoculated onto Sabouraud dextrose agar plates (BBL Microbiology Systems, Cockeysville, MD, USA). Identification of *Aspergillus* spp. was based on gross colony morphologies and microscopic pictures. Cornmeal agar (BBL Microbiology Systems) slide cultures were used to identify molds.

GM detection

The detection of GM antigen by the Platelia *Aspergillus* EIA test (Bio-Rad, Marnes-La-Coquette, France) was carried out according to the manufacturer's instructions. A sample was considered positive if the index was ≥1.5. All the positive samples were retested with a new sample obtained from the patient because of the known association of false-positive results with sample contamination and to avoid problems of lack of reproducibility. Two consecutive positive patient samples were required for the classification of suspected IA.

Statistical analysis

Sensitivity and specificity were calculated on both a per-patient and a per-test outcome basis. For the per-patient calculation, each patient was classified as positive if a positive test result was obtained, as determined for a range of optical density cut-off index criteria. Thus, sensitivity was calculated as the proportion of patients with a diagnosis of proven or probable *Aspergillus* infection. Similarly, specificity was determined by the proportion of patients with no positive test result. Cases classified as possible *Aspergillus* infection were not included in the analyses because this group contained a great deal of diagnostic uncertainty.

Results

Characteristics of the study population

A total of 189 patients who were subjected to tests for the detection of GM antigen during hospital stay were initially identified for evaluation. The data analysis was restricted to the group of patients meeting the criteria for proven or probable IA. Among these patients, 5 had proven IA, 9 had probable IA, and 149 patients did not have IA. The remaining 26 patients had possible IA. The clinical characteristics of the patients with proven or probable IA are shown in Table 1. The median age was 54 years (range, 12-76 years). There were 7 male and 7 female patients; 9 patients had hematological diseases, including lymphocytic leukemia in 4, acute myelogenous leukemia in 3, severe aplastic anemia in 1, and multiple myeloma in 1. Before GM assay, all of these patients had received cytotoxic

Table 1. Characteristics of 14 patients with proven and probable invasive aspergillosis (IA)

Patient	Age (years)	Gender	Primary disease or risk factor	Site of infection	IA classification	Platelia *Aspergillus* EIA test Positive/total number of tests	Days between start of antifungal therapy and Platelia *Aspergillus* EIA	Hospital stay (days)	Outcome
1	30	F	ALL, ChemoTx, HSCT	Lung	Proven	1/2	27	82	Mortality
2	74	M	Nil	Lung	Proven	1/1	24	73	Mortality
3	12	F	SAA, ChemoTx	Lung and skin	Proven	2/2	6	33	Mortality
4	48	F	ALL, ChemoTx	Sinus	Proven	0/1	42	109	Mortality
5	53	F	ALL, ChemoTx	Spine	Proven	1/3	6	59	Survived
6	28	M	AML, ChemoTx	Lung	Probable	1/2	8	58	Mortality
7	59	M	AML, ChemoTx	Lung	Probable	3/3	6	40	Survived
8	19	F	ALL, ChemoTx	Lung	Probable	0/2	2	69	Mortality
9	40	F	SLE, steroid	Lung	Probable	1/2	5	110	Survived
10	55	F	SLE, steroid	Lung	Probable	1/1	2	21	Mortality
11	63	M	MM, ChemoTx	Lung	Probable	1/1	3	22	Mortality
12	76	M	IPF, Steroid	Lung	Probable	0/2	4	25	Mortality
13	76	M	AML	Lung	Probable	1/1	28	79	Survived
14	55	M	Steroid	Lung	Probable	1/1	1	10	Mortality

Abbreviations: EIA = enzyme immunoassay; F = female; M = male; ALL = acute lymphocytic leukemia; ChemoTx = cytotoxic chemotherapy; HSCT = hematopoietic stem cell transplantation; SAA = severe aplastic anemia; AML = acute myelogenous leukemia; SLE = systemic lupus erythematous; MM = multiple myeloma; IPF = idiopathic pulmonary fibrosis

chemotherapy and 1 had undergone transplantation; 2 patients had systemic lupus erythematous and both had long-term steroid use; 6 patients had neutropenia during episodes of IA and all of them had underlying hematological diseases. The median duration of neutropenia was 25.5 days (range, 15-102 days). *Aspergillus fumigatus* was the most common pathogen in 7 patients, followed by *Aspergillus flavus* in 3 patients and *Aspergillus terreus* in 1 patient. The median duration of hospital stay was 58.5 days (range, 10-110 days). The mortality rate was 71.4%.

GM detection

A total of 304 samples were obtained from 189 at-risk patients. The test result was positive in 35 sera (25 patients) and negative in 269 sera (164 patients). The overall Platelia *Aspergillus* EIA test results in the different patient categories are summarized in Table 2. Of the 189 patients, 5 were diagnosed as proven IA. Of these patients, 1 had 2 consecutive serum samples with positive results and the other 3 had 1 positive GM assay. Among the 9 patients with probable IA, 15 GM assays were performed in all, and 7 had 1 (in 6 patients) or 3 consecutive (in 1 patient) positive test results. Among the 26 patients with possible IA, 1 patient had 3 serum samples with positive results and 4 patients had 1 positive GM result. For the remaining 149 patients, a thorough clinical evaluation and radiographic findings did not provide any evidence to support a diagnosis of IA. Nevertheless, 9 of these patients had at least 1 positive GM assay result among the total 20 tests,

Table 2. Platelia *Aspergillus* enzyme immunoassay test results of patients with proven, probable, or possible invasive aspergillosis (IA) or without a retained diagnosis of IA

Category	Platelia results (No. of patients)		
	Positive	Negative	Total
Proven IA	4	1	5
Probable IA	7	2	9
Possible IA	5	21	26
IA not retained	9	140	149
Total	25	164	189

including 3 patients with 2 and 6 patients with 1 positive result. Among the 9 patients, 5 had hematological malignancy, 2 had solid organ malignancy, 1 had interstitial lung disease, and 1 had bronchiectasis. All of them had severe pneumonia while taking the GM assay. Considering true positive as only those obtained for patients with proven or probable IA and true negatives as those in patients classified as not having IA, the sensitivity, specificity, and positive and negative predictive values of GM assay were 78.6%, 93.9%, 55.0%, and 97.9%, respectively.

Kinetics of GM-EIA indices during the clinical course of IA

GM-EIA index of patients with IA was not regularly monitored during the clinical course. In all, 3 patients with proven or probable IA who survived and had at least 2 GM assays; the indices seemed to gradually decrease after antifungal treatment (Fig. 1A). Only 5 of 11 patients who died had more than 1 positive GM assay, and 3 of these patients had increasing indices during the clinical course, but 2 had decreasing indices (Fig. 1B).

Discussion

Establishing a diagnosis of IA remains a challenge. Cultures may require days or weeks to grow, while the invasive procedures needed to obtain specimens for histopathological examination — still considered the "gold standard" of diagnosis — can rarely be performed on immunocompromised patients. Various approaches other than culture or tissue biopsy have been tried. Among them, GM-EIA is generally considered to be useful as a surrogate marker for invasive fungal infection [9-11].

The overall sensitivity of GM-EIA in this study was 78.6%, which is similar to the results reported by Sulahian et al — 76% with confirmed aspergillosis and 82.5% when probable and proven IA are considered together [8]. The disappointing sensitivity of the test in our proven IA cases could be attributed to fungal localization. One patient (case 4) who had acute invasive *Aspergillus* sinusitis with bone destruction was the only proven IA case with a negative GM-EIA result of 0.68. Although previous studies reported a better sensitivity (>90%), the selection of a low optical density index cut-off value for the GM-EIA may be one of the reasons [12-15]. If a lower cut-off value of 0.5, as approved by the US Food and Drug Administration [16], were to be applied to this study, the sensitivity would increase to 100%; however, the specificity would decrease to 65.7%. Thus, it may be worth adopting a lower cut-off value of ≥0.5 for the assay to be used as a screening tool.

Amphotericin B is known to suppress the expression of GM antigenemia in neutropenic rabbits with pulmonary aspergillosis [17]. Marr et al demonstrated that the sensitivity of GM-EIA is impaired by the administration of mold-active antifungal therapy [18]. In this study, all proven or probable cases had received anti-mold therapy before GM-EIA. Only 3 patients (cases 4, 8, and 12) had a GM-EIA index of <1.5. Among them, GM-EIA was carried out for cases 4 and 8 after treatment with amphotericin B for 42 and 2 days, respectively. GM-EIA test in case 12 was performed

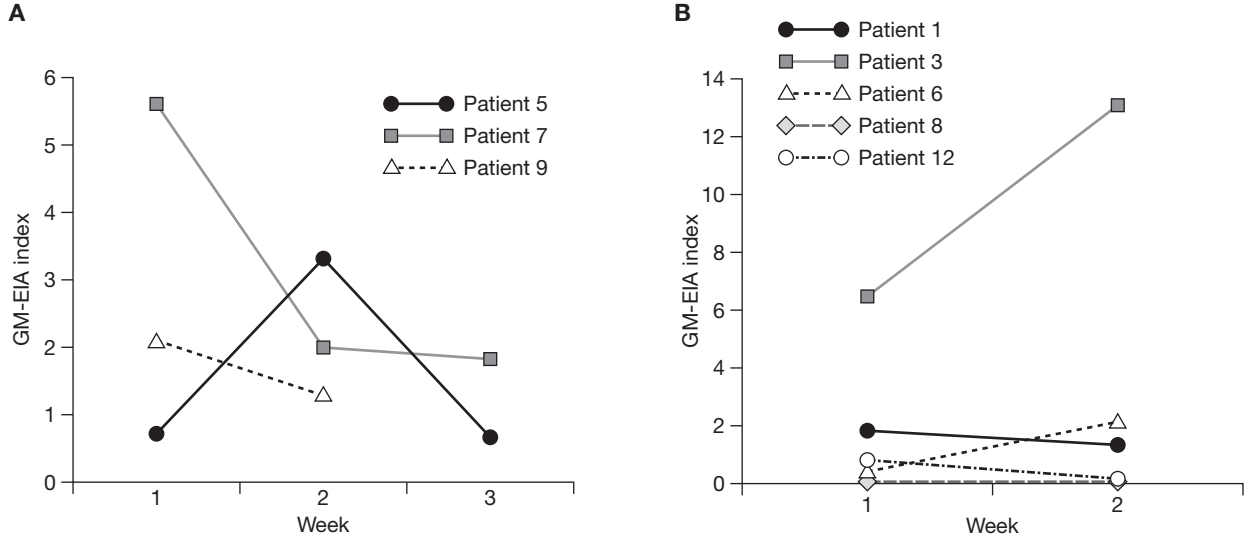

Fig. 1. Kinetics of galactomannan-enzyme immunoassay (GM-EIA) index in 3 patients who survived (A) and in 5 patients who died (B).

after treatment with voriconazole for 4 days. The other 11 cases had a GM-EIA index >1.5 after treatment with amphotericin B (in 10 patients) or caspofungin (in 1 patient) for a median duration of 6 days (range, 1-28 days). Although the number of cases is limited, the results suggested that the duration of antifungal drug exposure may not impact the detection of GM by this assay. The overall sensitivity was better than 52% in a previous report [18]. However, further study is needed to evaluate the impact of use of different mold-active antifungal drugs on GM-EIA assay results.

Some investigators have reported the usefulness of kinetics of GM levels due to its relation to clinical course [11,19]. As shown in Fig. 1A, a close relation was found between the kinetics of GM-EIA index and clinical course in the 3 surviving patients in this series. The GM-EIA index showed gradual decrease with the improvement of clinical conditions in these patients. In contrast, among the 5 patients who eventually died, only 3 patients (cases 3, 6, and 8) showed an increase in GM-EIA index and another 2 patients (cases 1 and 12) showed a decrease in values during the clinical course (Fig. 1B). A study employing more frequent measurement of the GM-EIA in a greater number of cases is needed to clarify the relation between the change in kinetics of GM indices and clinical course and outcome.

One of the problems observed in the detection of GM antigenemia is the existence of false-positive results. In our study, 9 patients without IA had at least 1 positive test result. Among these patients, 4 had received piperacillin-tazobactam and 1 had received amoxicillin-clavulanic acid at the time of detection. Two patients were being treated with hemodialysis at the time of GM-EIA. These findings are consistent with previous reports [20-22] that indicated treatment with piperacillin-tazobactam or amoxicillin-clavulanic acid and renal failure requiring dialysis as potential inducers of false-positive reactions. Besides, there were 3 patients who had candidemia at the time of detection of GM. Of these, 2 had received piperacillin-tazobactam and also had renal failure, but the remaining 1 patient had neither of these potentially confounding factors. Although *Pencillium marneffei* and spp. of the genera *Penicillium*, *Paecilomyces*, and *Alternaria* have been implicated in cross-reactions with Platelia *Aspergillus* EIA test [23,24], *Candida* has not been implicated. This case suggests that rising serum GM levels may not necessarily signify IA in patients with candidemia.

In summary, the Platelia *Aspergillus* EIA for GM antigen is a useful screening test for IA, especially if a lower cut-off value of >0.5 is adopted. This assay had good sensitivity even for patients receiving mold-active antifungal therapy in this series. Regularly monitoring the kinetics of GM-EIA indices can provide useful information due to its association with clinical course and outcome, but data interpretation should always be done cautiously because of the high frequency of false-positive results.

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