Comparison of Serum and Bronchoalveolar Lavage Galactomannan in Diagnosing Invasive Aspergillosis in Solid-Organ Transplant Recipients

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Abstract

Objectives: **This study sought to compare the sensitivities of serum galactomannan and bronchoalveolar lavage galactomannan in diagnosing invasive aspergillosis in solid-organ transplant recipients (lung and heart).**

Materials and Methods: **This study took place in the lung transplant center of the National Research Institute for Tuberculosis and Lung Disease. All patients with clinical and radiologic manifestations suggestive of pulmonary infection were included. Serum and bronchoalveolar lavage galactomannan were measured.**

Results: **Seventeen patients were included (lung, 15; heart, 1; heart-lung, 1). Probable or definite invasive aspergillosis was diagnosed in 9 patients. With a cutoff ≥ 0.5, serum galactomannan sensitivity and specificity for diagnosing invasive aspergillosis were 77.18% and 100%. Negative predictive value and positive predictive value were 80% and 100%. The sensitivity and specificity of bronchoalveolar lavage galactomannan for diagnosing invasive aspergillosis with cutoff of ≥ 0.5 was 100%.**

Conclusions: **Regarding the high levels of mortality and problems in diagnosing this disease, using bronchoalveolar lavage galactomannan could be a suitable option.**

Key words: *Aspergillosis, Fungal, Transplantation, Galactomanam, Bronchoalveolar lavage*

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Introduction

Invasive aspergillosis of the lung is a major cause of morbidity and mortality in immunocompromised patients.[1-4] Invasive aspergillosis occurs in 5% to 15% of patients with a solid-organ transplant.[5] The highest risk is with lung transplant recipients owing to colonization of the airways with *aspergillus* spp.[6, 7] Despite advances in treatment, mortality remains high (58% to 74%). Therefore, rapid diagnosis and timely treatment may decrease mortality.[8] Routine diagnostic measures such as smear and culture for fungi, radiology, and clinical symptoms have low sensitivity and specificity.[9-11]

Obtaining tissue samples is difficult owing to the condition of the patients as well as the risk of bleeding. Therefore, rapid tests (such as antigen detection) are promising options.[12, 13]

Galactomannan is a cell wall constituent of *aspergillus*, which elaborates during replication. The sensitivity and specificity of serum galactomannan in invasive aspergillosis are 61% to 71% and 89% to 93%.[5] Some studies have shown a higher sensitivity measuring galactomannan bronchoalveolar lavage fluid.[14]

This study sought to compare the sensitivity of serum galactomannan and bronchoalveolar lavage galactomannan for diagnosing invasive aspergillosis in solid-organ transplant recipients (lung and heart).

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Materials and Methods

This study took place from January 2009 to January 2010 at the lung transplant center of Massih Daneshvari Hospital, which is the primary hospital for heart and lung transplants in Iran. All patients who had clinical and radiologic manifestations suggestive of pulmonary infection were prospectively included. All protocols were approved by the ethics committee of the institution before the study began, and the protocols conformed to the ethical guidelines of the 1975 Helsinki Declaration. Written, informed consent was obtained from patients or their guardians.

Immunosuppressive regimen

The baseline regimen for all patients consisted of cyclosporine, mycophenolate mofetil, and prednisolone. In some cases, cyclosporine was replaced by tacrolimus.

Prophylactic regimen

All patients received meropenem plus vancomycin for 72 hours after the operation. Ganciclovir was prescribed for 3 months after the transplant. Itraconazole was administered for all lung transplant patients during the first 3 months. Cotrimoxazole was used for at least the first year after the transplant in all patients.

Mycologic studies

Sputum or bronchoalveolar lavage fluid was homogenized and subjected to direct microscopy using 10% potassium hydroxide and lactophenol cotton blue mounts. Fungi cultures were done by plating clinical specimens on Sabouraud Dextrose Agar and incubating the plates at 37ºC for 3 to 6 days. *Aspergillus* species were identified by their culture characteristics and morphologies.[15]

Galactomannan detection

The *aspergillus* galactomannan antigen was detected in serum or bronchoalveolar lavage by direct double-sandwich enzyme-linked immunosorbent assay (platelia *aspergillus*; Bio Rad, Marnes, La Coquette, France). Serum or bronchoalveolar lavage was heated at 100ºC for 3 minutes with a solution of ethylenediaminetetraacetic acid, and then centrifuged for 10 minutes.

Fifty microliters of supernatant and 50 μL of conjugate were incubated in the monoclonal antibody-coated microplate for 90 minutes at 37ºC. After 5 washings, the platelia were incubated (in the darkroom at a temperature of 18°C to 25ºC for 30 ± 5 min) with a substrate chromogen solution containing tetramethylbenzidine. The reaction was stopped with 100 μL sulfuric acid, and the plates were read in an enzyme-linked immunosorbent assay reader (Awareness Technology, Inc. Palm City, FL, USA) at 450 nm (reference 620 nm).

Classification of invasive aspergillosis was done based on the Infectious Diseases Society of America guidelines for aspergillosis. Three categories of "definite," "probable," and "possible" were defined.[16] From a practical point, definite and probable cases require prompt antifungal treatment.

Results

In the lung transplant recipients, underlying pulmonary diseases included bronchiectasis (6), lung fibrosis (5), chronic obstructive pulmonary disorder (3), and bronchiolitis obliterans (1).

The heart transplant patient had advanced cardiomyopathy, and the heart-lung transplant was done on a patient with congenital heart disease. The mean age of the patients was 34.58 ± 1.5 years (range, 12-50 y).

Probable or definite invasive aspergillosis was diagnosed in 9 patients. In 8 patients, invasive aspergillosis was not confirmed and alternative pathogens were isolated. *Candida* was isolated in 7 patients, and no pathogen was recovered in 1 case.

With a cutoff of ≥ 0.5, serum galactomannan sensitivity and specificity for diagnosing invasive aspergillosis were 77.18% and 100%. Negative predictive value and positive predictive value were 80% and 100%. The sensitivity and specificity of bronchoalveolar lavage galactomannan for diagnosing invasive aspergillosis with a cutoff of ≥ 0.5 were 100%. A summary of patients with invasive aspergillosis is shown in Table 1.

Discussion

Although most people are frequently exposed to *aspergillus*, infections caused by the fungus rarely occur in an immunocompetent patient.[2] Invasive aspergillosis is the most-common life-threatening opportunistic invasive mycosis in immune-compromised patients.[5] The incidence of

Table 1. Characteristics of patients with invasive Aspergillosis.

Patient	Age/sex	Underlying disease	Type	Onset posttransplant	Definite probable	GM (BAL)	GM (serum)	Outcome
1	20/M	Bronchiectasis	Double lung	13 mo	Probable	0.5	0.3	Dead
2	21/M	CF	Double lung	28 mo	Probable	3.5	0.38	Alive
3	50/M	COPD	Right lung	18 mo	Probable	3.5	0.8	Alive
4	21/M	Bronchiectasis	Double lung	1 mo	Probable	2.5	1.5	Alive
5	22/M	Bronchiectasis	Double lung	1 mo	Probable	4.5	0.7	Alive
6	24/M	Bronchiectasis	Double lung	15 mo	Probable	4.9	3.5	Alive
7	52/M	COPD	Right lung	3 mo	Probable	1.4	0.8	Alive
8	22/M	Bronchiectasis	Left lung	15 mo	Probable	1.32	0.9	Alive
9	59/M	Cardiomyopathy	Heart	1 mo	Definite	0.5	0.62	Alive

Abbreviations: BAL, bronchoalveolar lavage; CF, cystic fibrosis; COPD, chronic obstructive pulmonary disorder; GM, galactomannan; M, male

aspergillosis in patients with bone marrow transplant is high, and the incidence of invasive aspergillosis among solid organ transplant recipients is lower.[11] Despite antifungal treatments, mortality remains high (50% to 70%).[6]

The definitive diagnostic method is positive culture yielding aspergillosis from lung secretions or detecting fungal hyphae in biopsy specimens. There are some disadvantages for both methods. Culture for fungal detection has a low level of sensitivity; furthermore, the biopsy is invasive and potentially harmful. Some alternate methods (such as galactomannan measuring and Beta-glucan) seem useful in diagnosing invasive aspergillosis.[9] Galactomannan detection in serum is simple; however, clinical specificity is estimated at 89% to 93% and sensitivity is 61% to 71%. The specificity and sensitivity in patients with any transplant appears to be lower, and varying reports exist.[5] For example, patients with lung transplants have been mentioned to have a 30% sensitivity and a 94% specificity.[5]

It seems that measuring galactomannan in bronchoalveolar lavage has a higher sensitivity.[5] Another study showed that among 81 patients with solid-organ transplant, the sensitivity and specificity of detecting galactomannan in bronchoalveolar lavage was 100% and 90% in diagnosing invasive aspergillosis (cutoff > 1).[8]

False-positive cases are reported frequently, especially in patients with lung transplant, owing to a high rate of *aspergillus* colonization in these patients.[8]

Another study of 116 lung transplant cases reported 60% sensitivity and 95% specificity for bronchoalveolar lavage galactomannan measuring (cutoff > 0.5).[7] In comparison, our study had a higher sensitivity and specificity with both methods (serum and bronchoalveolar lavage galactomannan measuring) in diagnosing invasive aspergillosis.

Two different studies have reported a high level of false positives for bronchoalveolar lavage galactomannan owing to colonization of *aspergillus* in the airway.[7, 8] In our study, we did not have this problem.

We have 2 explanations for this finding: First, we selected patients with clinical and radiologic findings compatible with pulmonary infections. However, in other studies, patients with routine bronchoscopy also were included. Second, the prophylactic antibiotic regimen in our study consisted of meropenem, which is not among the antibiotics that can cause a false-positive galactomannan test.

The problem in all these studies (on transplant patients) is the incidence of invasive aspergillosis. In the aforementioned studies, there were only 5 to 6 invasive aspergillosis cases.[7, 8] In our study, we had 9 cases. However, our total sample size was small. Another important finding in our study was the low rate of mortality. This may be because of the earlier diagnosis and effective treatment. Our treatment was based on the combination of voriconazole and caspofungin in invasive aspergillosis.

Regarding the high levels of mortality and problems in diagnosing this disease, using bronchoalveolar lavage galactomannan could be a suitable. Identifying galactomannan > 0.5 in a bronchoalveolar lavage specimen could be used as an effective standard for starting antifungal therapy.

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