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Detection of galactomannan in bronchoalveolar lavage of the intensive care unit patients at risk for invasive aspergillosis

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Abstract

Background and Purpose: Invasive aspergillosis (IA) is one of the most common life-threatening fungal infections among the critically ill patients including intensive care unit (ICU) patients. Delayed diagnosis and therapy may lead to poor outcomes. Diagnosis may be facilitated by a test for molecular biomarkers, i.e. detection of galactomannan (GM) antigen based on enzyme immunoassay, which is of increasing interest in the clinical settings for the diagnosis of IA. In the present study, we assessed GM testing of bronchoalveolar lavage (BAL) fluid as a tool for early diagnosis of IA among ICU patients who were at risk for developing IA.

Material and Methods: A prospective study was performed in ICU patients with underlying predisposing conditions for IA between August 2010 and September 2011. BAL samples for direct microscopic examination, culture, and GM detection were obtained once or twice weekly. GM in BAL levels was measured using the Platellia Aspergillus EIA test kit. According to modified European Organization for the Research and Treatment of Cancer/ Mycoses Study Group (EORTC/MSG) criteria, patients were classified as having probable or possible IA.

Results: Out of 43 suspected patients to IA, 13 (30.2%) cases showed IA. According to the criteria presented by EORTC/MSG, they were categorized as: 4 cases (30.8%) of possible IA and 9 (69.2%) of probable IA. Out of 21 BAL samples from patients with IA, 11 (52.4%) had at least one positive BAL GM index. Using a cutoff index of 0.5, the sensitivity and specificity, positive and negative predictive values of GM detection in BAL fluid were 100%, 85.7%, 65.7% and 96%, respectively. The sensitivity and specificity was 73% and 92.7% at cutoff ≥ 1.0, respectively. In 6 of 13 IA cases, BAL culture or direct microscopic examination remained negative, whereas GM in BAL was positive.

Conclusion: Our data have revealed that the sensitivity of GM detection in BAL was better than that of conventional tests. It seems that GM detection in BAL is beneficial to establish or exclude the early diagnosis of IA in ICU patients.

Keyword: Invasive Pulmonary Aspergillosis, galactomannan, Bronchoalveolar Lavage, Intensive Care Units

Introduction

Invasive aspergillosis (IA) is one of the most common life-threatening fungal infections that has emerged as an important cause of morbidity and mortality in immunocompromised patients that ranges from 70% to 90% in one year [1]. IA is due to ubiquitous *Aspergillus* species that invade from the lungs into the adjacent organs if the immune system is compromised. Recently several reports have revealed a rising incidence of IA in critically ill patients admitted to the intensive care unit (ICU) in otherwise healthy individuals [2-5]. The occurrence of IA in the ICU usually entails a poor prognosis, despite major recent improvements in the diagnosis and treatment of IA in patients with hematologic diseases [6]. Early diagnosis of IA is very important and requires diagnostic tools validated in the ICU

population that show positive results in an early phase of the infection [7]. In IA, the clinical manifestations and symptoms are generally non-specific, mechanical ventilation makes it difficult to interpret clinical signs, and radiological diagnoses are clouded by underlying lung pathologies [8]. The significance of positive results of culture or positive findings of a direct microscopic examination of a respiratory specimen is greatly uncertain, because they have low sensitivity (50%) and specificity (20%–70%), depending on whether the patient is immunocompromised [9,10]. Histopathological examination as the golden standard method is not often practicable because of the patient's status that prohibits invasive procedures [11]. Therefore due to the restrictions of the aforementioned diagnostic tools, a non-culture based method, which can detect a biomarker such as galactomannan (GM) antigen, has been developed. GM is a cell wall polysaccharide component released during growth and invasion of *Aspergillus* in tissue that is detectable in patients with invasive aspergillosis [12]. Studies showed a sensitivity of 61% to 71%, with a specificity of 89% to 93% for the detection of GM in serum samples [13]. Recently, detection of GM in BAL fluid has been considered as a diagnostic approach for IPA by some authors [14-16]. On the other hand, it has not well established a consensus on optical density (OD) cut-offs in BAL samples by ELISA method for the diagnosis of IA.

According to our search in international databases, there are no available data on GM detection in BAL samples among ICU patients in the Middle East. In the current study, we aimed to assess GM detection in BAL fluid with Platelia *Aspergillus* GM EIA as an early diagnostic tool among ICU patients who were at risk for developing IA.

Material and Methods
Patients
The Ethics Committee of Mazandaran University of Medical Sciences (code: 88-6-4) approved this research and the written informed consent was obtained from the patient or next of kin. We reviewed all adult patients suspected to have IA (host factors and clinical features) as defined by European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) [17] who hospitalized in the ICU of the University Hospitals in Mazandaran, Iran from August 2010 to September 2011. BAL samples were collected by pulmonologists twice weekly thereafter if feasible. Patients using piperacillin/tazobactam at the time of bronchoscopy and prolonged use (more than 1 week) of any antifungal drugs were excluded. Briefly, the lobe bronchus in which consolidation was imaged by chest radiograph or chest CT scan was wedged, and 20 to 50 ml of 0.9% sterile saline solution at room temperature was instilled with a syringe through the working channel of the bronchoscope. The total volume of saline solution instilled into the lung was typically 50 ml to 100 ml of BAL fluid was recovered. Subsequently, the obtained BAL fluid was sent directly unprocessed on dry ice to the department of Medical Mycology at Mazandaran University of Medical Sciences, Sari.

Laboratory work
A total of sixty six BAL fluid samples were collected. BAL samples were analyzed by direct microscopic examination using CalcoFlour White staining, fungal culture and GM detection. Cultures for fungi were performed by inoculating clinical specimens onto Sabouraud glucose agar at 30°C for 10 days. The fungi were identified by standard mycological techniques based upon gross cultural and microscopic morphology.

Platelia Aspergillus ELISA
The Platelia *Aspergillus* GM EIA (Platelia *Aspergillus*; Bio-Rad, Edmonds, WA) was performed for GM detection according to the manufacturer's instructions. Briefly, 100 µl of the Platelia treatment solution was added to 300 µl of the BAL specimen, which was then heated at 100°C for 3 min, followed by centrifugation at 10,000 g for 10 min. Then, 50 µl of the supernatant and 50 µl of the horseradish peroxidase-labeled monoclonal

antibody (EBA-2) were incubated at 37°C in antibody-precoated microplates for 90±5 min. The plates were washed 5 times, and then were incubated with 200 μl of substrate chromogen reaction solution for 30±5 min at room temperature in the dark. The reaction was stopped by the use of 100 μl sulfuric acid, and after 30 min, the plates were read at an optical density (OD) of the sample to that of the threshold control samples at 450 nm with a reference filter of 620 nm. An OD index of ≥1.0 in two consecutive samples was considered positive. All positive samples were rechecked and only if the repeat test was also positive they have take into accounted positive.

Case and definition of positive results

The Patients with IA were classified as having probable or possible IA on the basis of modified EORTC/MSG criteria [17]. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated according to the proportion of patients with true and false positive and negative tests. Calculations were also performed based on the certainty of IA.

Results

Table 1 summarized and characterized the patients who were enrolled in this study. According to modified EORTC/MSG criteria, out of 43 patients, 4 cases (30.8%) were classified as possible IA and 9 (69.2%) as probable IA. In this present study, we were not able to define a proven IA case because there was an explicit refusal of the family to do biopsy or autopsy. Out of 21 BAL samples obtained from 13 patients with probable IA, 76.2% was positive for GM. Of these, 53.8% and 77% of samples had positive results in culture and direct microscopic examination methods, respectively (Table 2).

In 6 cases, culture or direct microscopic examination on BAL samples remained negative, whereas GM detection was positive. All patients with IA had at least one positive BAL GM (OD index ≥ 0.5 and > 1.0). Out of 45 BAL samples from patients without IA, 43 (95.5%) had negative GM results (Figure 1).

Table 3 shows the sensitivity, specificity, positive and negative predictive values of different BAL GM index. A cut off ≥ 0.5 yielded a 100% of sensitivity and 96% of negative predictive value (NPV) with relatively low specificity and positive predictive value (PPV). On the other hand, a cut off >2 improve of specificity and PPV.
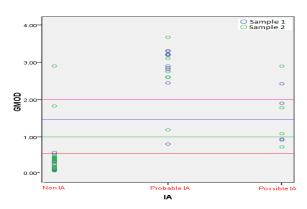

Figure 1. Distribution of BAL GM results.

Table 1. Characteristics of studied patients

Characteristics		Probable IA (n=9)	Possible IA (n=4)	Non IA (n=30)	All (n=43)
Mean of age (years)		47.8	62	58.4	56.5
Sex, male, (%)		6 (66.6)	2 (50)	16 (53)	24 (58.8)
ICU length of stay (days)		7.3	9.75	25	19.6
Mechanical ventilation (days)		6.7	6.25	22	18.2
No. of deaths (%)		7 (77.8)	2 (50)	8 (26.7)	16 (37)
Underlying disease	Neutropenia (%)	7 (77.8)	0	0	8 (18.6)
	Hematologic (%)	5 (55.6)	2 (6.7)	2 (6.7)	9 (30)
	COPD (%)	1 (11.0)	1 (3.3)	1 (3.3)	3 (7)
	Solid cancer (%)	2 (22.0)	5 (16.7)	5 (16.7)	8 (18)
	Diabetes	0	5 (16.7)	5 (16.7)	6 (14)
	Other	0	25 (83.3)	25 (83.3)	25 (58)

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; IA = invasive aspergillosis; ICU =intensive care unit.

Table 2. BAL galactomannan, direct examination and culture results in patients.

| | | Percentage of patients | | |
		IA	No IA	Total
BAL GM[1]	Positive	84.6	6.6	30.2
	Negative	15.3	93.3	69.7
BAL direct examination	Positive	77	36.6	48.8
	Negative	23	63.3	51.1
BAL culture	Positive	53.8	20	30.2
	Negative	46	80	69.7

Definition of abbreviation: BAL= bronchoalveolar lavage; GM = galactomannan.[1]Cutoff value for positivity: 1.0 ng/ml

Table3. Sensitivity, specificity and predictive values for some selected cutoff GM index of patients with probable and possible IA as a standard (%).

GM index	Sensitivity	Specificity	PPV	NPV
≥0.5	100	85.7	65.7	96
≥1.0	73	92.7	89	87.3
≥ 1.5	66	96.8	94	87
≥ 2.0	64	98	96	85

Definition of abbreviation: PPV= positive predictive value; NPV= negative predictive value; GM = galactomannan

Discussion

Detections of GM in BAL samples can be consider as a method, which facilitate the early diagnosis of invasive aspergillosis-a life threatening disease in immunocompromised patients. In spite of several disadvantages, BAL or serum GM detection remains a powerful technique in the diagnosis and management of patients at high risk of invasive aspergillosis [18]. To our knowledge, this is the first study to evaluate the utility of measuring BAL galactomannan in ICU patients in Iran. Recently, several prospective studies in ICU patients reported sensitivities, specificities and predictive values above 90% for GM detection in BAL samples [4, 14, 19]. Our results indicated that BAL GM detection with higher sensitivity (100 %) and specificity (85.7 %) at a cutoff ≥ 0.5 compared to conventional methods for the diagnosis of IPA in at-risk patients in ICU. This observation is similar to those described by previous studies [14, 19]. The high sensitivity of GM detection in the BAL may reduce the specificity due to false positive results. However, many sources of false positivity including piperacillin – tazobactam or amoxicillin – clavulanate are frequently and remarkably encountered in the ICU [20], the specificity was still above 85%. In our study, BAL GM detection was performed early, promptly after CT and before the start of antifungal treatment. Verweij et al, and Salonen et al, suggested that antifungal therapy can explain the somewhat lower sensitivities of GM detection in BAL fluid [21, 22].

Our study had several limitations; rare case of IA and small sample size which may have limited our power to detect differences between the groups, bronchoscopy procedures is not usually performed in the evaluation of IA in ICU patients due to the critical conditions of patients, and unless with abnormal respiratory signs at CT finding and autopsy process from patients suspected IA, has not been reported cases of proven IA. Moreover, OD measurements are dependent on the concentration of GM antigen, which being dependent on several factors such as the area that is washed, the amount of saline instilled during bronchoscopy procedure, and the amount of fluid retrieved. In our study, instilled either 20 or 50 ml each time, this difference may add variability to the results. The amount of sterile saline instilled varied significantly (40 to 150 ml) between studies [15, 16, 23, 24] suggesting that the results although similar may not be comparable or extrapolated to other centers. Therefore, a standardized method of collection should also be established before results can be comparable between centers. Finally, we cannot provide data on the influence of antifungal therapy on the performance of GM detection in BAL fluid.

Conclusion

Since the diagnosis of IA is a big challenge, early diagnosis is crucially important because early antifungal therapy improves surviva1. Our data have revealed that the sensitivity of GM detection in BAL was better than that of conventional tests. It seems that GM detection in BAL is beneficial to establish or exclude of early diagnosis of IA in ICU patients. Therefore, a prospective screening of BAL GM is an essential role for early diagnosis of IA,

especially when it is combined with other diagnostic tools. The validity of the data needs to be confirmed in other ICUs and examine various aspects in ICU Patients.

Acknowledgments

Authors' contributions

S.K. performed all tests and wrote the draft. MT.H. designed and managed the research, and edited the final manuscript. M.A. and MR.H. referred the patients. H.B. helped to analysis of data.

Conflicts of interest

All authors report no potential conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Financial Disclosure

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