No. 147

Galactomannan detection in Bronchoalveolar lavage fluids: a diagnostic approach for fungus ball in patients with pulmonary tuberculosis?

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Introduction

The lung cavitary lesions left after the treatment of pulmonary tuberculosis (PTB) could increase the risk of chronic pulmonary aspergillosis (CPA) and fungus ball (fungoma) [1]. It is suggested that 11-20% of treated PTB patients with cavitary lesions develop aspergilloma [2]. Various fungal genera can be involved in the formation of a fungus ball. However, *Aspergillus* species are considered as the most common cause of disease [3]. *Scedosporium boydii*, *Fusarium* species, and the species related to *Mucorales* accounted for less than 5% in the occurrence of fungus ball [4- 6]. The radiological findings play a significant role in the diagnosis of fungus balls. Given that radiological findings cannot establish an accurate diagnosis of fungoma and the obtained results might overlap with some other pulmonary diseases (e.g., neoplasm), it is essential to use mycological methods to confirm fungoma diagnosis. On the other hand, culture of respiratory samples including sputum or bronchoalveolar lavage (BAL) has a low sensitivity [7, 8]. Therefore, non-culture based diagnostic methods, including serum specific IgG against *Aspergillus*, with 93.4% sensitivity and 98.7% specificity, are widely used as a diagnostic approach [2, 8, 9]. According to the limited published data [10, 11], it is suggested that the GM detection in BAL fluid can be also considered as a diagnostic approach for fungus ball. However, further data would be need to validate the approach to use in clinical setting. **Therefore, in this present study we aimed the diagnosis of fungus balls in patients with PTB referring to the Reference Center for TB and Pulmonary Diseases of Iran and the evaluation of GM levels in the BAL samples for fungus ball diagnosis.**

Material and Methods

•Patients

This retrospective study was conducted on a total of 110 PTB patients (60 with active PTB and 50 with previous history of PTB). The study population were selected from the patients referred to the Reference Center for TB and Pulmonary Diseases of Iran, Tehran, Iran, during 2017-2019. The Ethics Committee of Mazandaran University of Medical Sciences (code: IR.MAZUMS.REC.96.2938) approved the research and the written informed consent was obtained from the patients.

•Radiological Evaluations

Radiological evaluation was performed to detect cavitary lesions and suspicion of the fungal mass along with air crescent sign in both lung lobes using thoracic computed tomography (CT) scan and/or chest X-ray (CXR). Other symptoms, such as nodules, pleural effusion, and fibrosis were also recorded.

•Lab diagnoses

The BAL samples were collected using a fiberoptic bronchoscope in included patients. Each BAL samples were analyzed for GM detection, direct microscopic examination (DME) mounted with 20% potassium hydroxide and fungal culture on Sabouraud dextrose agar (SDA). The grown colonies were independently sub-cultured onto SDA and were then identified at species level by macroscopic and microscopic characteristics of each colonies.

The identification of *Aspergillus* species obtained from patients with fungus ball were confirmed by PCR-sequencing of β-tubulin genes.

•GM Platelia *Aspergillus* assay

The Platelia *Aspergillus* GM EIA (Bio-Rad Laboratories, Marnes-la-Coquette, France) was used to detection of GM on BAL fluid specimens, according to manufactures manual, using 300µl of the BAL sample. Positive and negative controls ELISA kit were included in each test. The results were recorded as GM index ≥ 0.5 and GM index ≥ 1.

•Histopathology

The tissue samples were taken from the pulmonary lesion of patients suspected to fungus ball. The samples were assessed by staining of the sections via H&E staining technique.

•Data analysis

Data were analyzed using SPSS® 22.0 (SPSS Inc., Chicago, IL, USA). The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for each possibility of the cut-off values of GM test were calculated by Fisher Exact test and Chi-squared test (χ2 test). *P*-value less than 0.05 was considered as statistically significant. The optimal cut-off for BAL GM testing was determined by receiver operating characteristic (ROC).

Results

A total of 110 PTB patients (60 with active PTB and 50 with previous history of PTB) were included. The age range of patients was 16-87 years with a mean age of 56.5 years. Of 110 patients, 40 (36.3%) and 45 (40.9%) cases were positive for septate hyphae in direct microscopic examination and growth of *Aspergillus* species in culture of BAL samples, respectively. *A. flavus* complex (35, 38.9%) was the most common followed by, *A. fumigatus* complex (20, 22.2%) and *A. niger* complex (19, 21.1%).

Out of 110 patients with PTB, 9 (8.18%) patients showed fungus ball, all with old PTB. Histopathology demonstrated fungus hyphae in biopsied tissue from all 9 patients. The patients with positive results in histopathology and culture for *Aspergillus* in BAL samples were considered as aspergilloma (4, 44.4%) and others as fungus ball (5, 55.5%). The molecular approach on isolated *Aspergillus* species in aspergilloma cases confirmed the isolates as *A. flavus* (2, 50%), *A. fumigatus* in (1, 25%), and A. *ochraceus* (1, 25%).

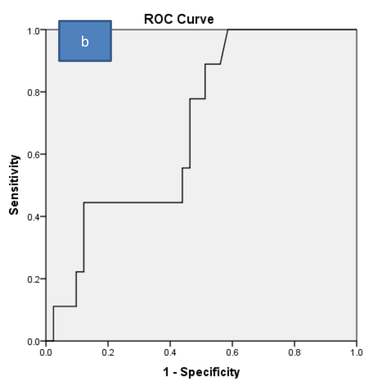
Table 2. The sensitivity, specificity, and positive and negative predictive values for two cut-off GM indexes ≥ 0.5 and ≥ 1 in patients with fungus ball and aspergilloma

	Fungus ball in old TB		Aspergilloma in old TB	
GM index	≥ 0.5	≥ 1	≥ 0.5	≥ 1
Sensitivity	100	55.6	100	75
Specificity	41.5	58.5	37	58.7
PPV	27.3	22.7	12.1	13.6
NPV	100	85	100	96
Correlations Coefficient (Phi and Cramers)	0.34	0.11	0.21	0.18
Likelihood ratios	8.46	0.59	3.5	1.27
AUC	0.7	0.63	0.75	0.58
p- value	0.02	0.48	0.28	0.3

GM, galactomannan; PPV, Positive Predictive Value; NPV, Negative Predictive Value; AUC, Area under the curve

Table 1. Distribution of galactomannan antigen level in patients with active and old tuberculosis, patients with fungus ball and patients without fungus ball.

Patients (Numbers)		GM Mean ± SEM	GM Median	Mann-Whitney U test	
				Mean Rank GM	p- value
Patients(93)	Old TB (50)	1.36 ± 0.21	0.8	52.57	0.03
	Active TB (43)	0.087 ± 0.17	0.53	40.52	
Fungus ball in old TB (9)	NO (41)	1.2 ± 0.22	0.71	23.62	0.05
	YES (9)	2.04 ± 0.56	1	34	
Aspergilloma in old TB (4)	NO (46)	1.24 ± 0.2	0.71	24.42	0.07
	YES (4)	2.67 ± 1.07	2.33	37.88	



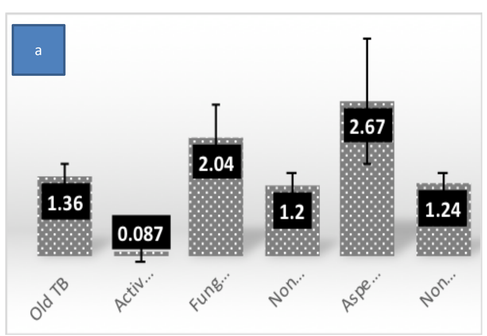


Fig 1. a, Box plots of the mean ± SEM galactomannan index values between BAL samples of patients with old TB, active TB, fungus ball in old TB, and non- fungus ball and Aspergilloma in old TB and non Aspergilloma; **b,** receiver operating characteristic (ROC) curve of BAL GM in 9 patients with fungus ball comparing sensitivities and specificity of GM index ≥ 0.5.Area under the curve (AUC) =0.7.

GM analysis in BAL samples

Out of 110 included patients, 93 (43 with active PTB and 50 with old PTB) cases were analyzed for BAL GM index. Of these 93 patients, 55 (59.1%) and 34 (36.5%) were positive for GM index ≥ 0.5 and ≥ 1 in BAL samples, respectively. Out of 50 patients with old PTB, 33 (66.0%) and 23 (46.0%) had GM index ≥ 0.5 and ≥ 1 in BAL samples, respectively. Fig 1a show the distribution of GM antigen level in patients with active and old PTB, patients with fungus ball and patients without fungus ball. According to BAL's GM index ≥ 0.5, all patients with fungus balls had positive results.

The Mean ± SEM of GM antigen in BAL samples of patients with active PTB and old PTB were 0.087±0.17 (mean rank: 40.52, median: 0.53) and 1.36 ± 0.21 (mean rank: 52.57, median: 0.8), respectively (Table 1). The Mann-Whitney U test showed a significant difference between two groups (*p- value*, 0.03). The Mean ± SEM of GM antigen in the BAL samples of 9 patients with fungus ball and 41 patients without fungus ball were 2.04±0.56 (mean rank: 34, median: 1) and 1.2 ± 0.22 (mean rank: 23.62, median: 0.71), respectively. There was a statistically significant difference between two groups (*p- value* = 0.05). The Mean ± SEM of GM antigen in the BAL samples of 4 patients with aspergilloma and 46 patients without aspergilloma were 2.67±1.07 (mean rank: 37.88, median: 2.33) and 1.24 ± 0.2 (mean rank: 24.42, median: 0.71), respectively. There was no statistically significant difference between two groups (*p- value*, 0.07) (Table 1).

The sensitivity, specificity, positive, negative predictive value, correlations coefficient (Phi and Cramers), likelihood ratios and AUC for GM detection (index ≥ 0.5) in BAL samples of patients with fungus ball were 100%, 41.5%, 27.3%, 100%, 0.34%, 8.46% and 0.7 (*p- value*, 0.02) and those of GM index ≥ 1 were 55.6%, 58.5%, 22.7% , 85%, 0.11%, 0.59 % and 0.63 (*p- value*, 0.48), respectively (Fig 1b and Table 2).

The sensitivity, specificity, positive, negative predictive value, correlations coefficient (Phi and Cramers), likelihood ratios and AUC for GM detection (index ≥ 0.5) in BAL samples of patients with aspergilloma were 100%, 37%, 12.1%, 100%, 0.21%, 3.5% and 0.75 with (*p- value*, 0.28) and those of GM index ≥ 1 were 75%, 58.7%, 13.6% , 96%, 0.18%, 1.27% and 0.58, respectively (*p-value* = 0.3) (Table 2).

Conclusion

According to our results the detection of GM in BAL samples could be considered as an approach for diagnosis of fungus ball in patients with underlying condition including TB. Our data have also supported that the optimal cut-off value for BAL GM detection is index ≥ 0.5 in PTB patients suspected to fungus ball. However further studies with a larger samples are needed in order to assess the possible use of GM detection for diagnosis of fungus ball or aspergilloma in suspected patients.

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