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

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Introduction
The lung cavities left after the treatment of pulmonary tuberculosis (PTB) could increase the risk of chronic pulmonary aspergillosis (CPA) and fungus ball (fungomia) [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 this disease. 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 fungus ball and the obtained results might overlap with some other pulmonary diseases (e.g., neoplasm), it is essential to use mycological methods to confirm fungusia 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 [7, 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.MAZU.MREC.96.2938) approved the research and the written informed consent was obtained from the patients.

Radiological evaluation
Radiological evaluation was performed to detect cavitory lesions and suspicion of the fungus ball along with air crescent sign both in 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 sample was 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 gene.

GM Plateia Aspergillus assay
The Plateia Aspergillus GM EIA (Bio-Rad Laboratories, Marnes-la-Coquette, France) was used to detect GM on BAL fluid specimens, according to manufacturers 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 (q2 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 TB. Histopathologically demonstrated fungus hyphae in biopsy 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 1: Distribution of galactomannan antigen titer in patients with active and old tuberculosis with positive and negative results of galactomannan assay in Bronchoalveolar lavage fluids

<table>
<thead>
<tr>
<th>Patient (Nos)</th>
<th>GM Mean ± SEM</th>
<th>GM Median</th>
<th>Mann-Whitney U test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive GM (Cr)</td>
<td>0.16 ± 0.62</td>
<td>0.8</td>
<td>0.03</td>
</tr>
<tr>
<td>GM Positive (Cr)</td>
<td>0.12 ± 0.06</td>
<td>0.71</td>
<td>24.42</td>
</tr>
<tr>
<td>GM Negative (Cr)</td>
<td>2.67 ± 0.07</td>
<td>2.33</td>
<td>37.88</td>
</tr>
</tbody>
</table>

In conclusion, we recommended using BAL GM index ≥ 0.5 as a diagnostic tool for fungus ball in PTB patients. However, further studies including 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.