INVASIVE ASPERGILLOSIS IN INTENSIVE CARE UNIT PATIENTS
IN IRAN

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Summary: We assessed the intensive care unit (ICU) patients for Invasive aspergillosis (IA) with culture and non-culture based diagnostic methods from Iran. Thirty-six ICU patients with underlying predisposing conditions for IA were enrolled in the study. Sixty-eight Bronchoalveolar lavage (BAL) samples were collected by bronchoscope twice a weekly. BAL samples were analyzed by microscopic examination, fungal culture and galactomannan (GM) detection. The Platelia Aspergillus GM EIA was used to quantify GM indices. Samples with a BAL GM index ≥1 were considered as positive for GM. Patients were classified as having probable or possible IA. Out of 36 suspected patients to IA, 36.1% of cases showed IA which were categorized as: 4 cases of possible IA and 9 of probable IA. 76.2% of BAL samples were positive for GM. From 13 patients with IA, 11 (84.6%) had at least one positive BAL GM index. Of these patients, 9 (81.8%) showed probable IA. The main underlying predisposing conditions were neutropenia (53.8%) and COPD (30.8%). Our study has indicated that COPD must be considered as one of the main predisposing condition for occurrence of aspergillosis in ICU patients. Our data have also revealed that GM detection in BAL samples play a significant role to IA diagnosis.

Key word: Invasive aspergillosis; ICU; GM detection; BAL

Introduction

*Aspergillus* is one of the most prevalent airborne fungi both in indoor and outdoor environment. Our previous studies have also shown that the equipment and medical devices, potted plant and water can be a significant source for *Aspergillus* distribution in hospitals (17–19). Inhalation of *Aspergillus* conidia can give rise to various clinical conditions, depending essentially on the host’s immunological status (22). Invasive aspergillosis (IA) is one of the most common life-threatening fungal infections among critically ill patients including intensive care unit (ICU) patients. In the last two decades the infections caused by *Aspergillus* species have increased and *Aspergillus* is now the most common cause of systemic fungal infections in patients undergoing allogeneic bone marrow transplantation and those with acute leukaemia undergoing intensive chemotherapy (5, 26, 31).

Recent investigations have shown that IA must be considered as an emerging and mortal infectious disease in ICU patients even in the absence of an apparent predisposing immunodeficiency (24, 25). In addition, several new publications have also indicated that frequency of IA in the ICU has increased (13, 27, 30). On the other hand, IA in ICU patients is associated with prolonged hospital stay, and increased cost (30). The diagnosis of IA is still a great challenge in the ICU, and it is often made late in the course of the infection because of clinical manifestations are usually non-specific, mycological cultures are difficult to interpret or fungal growth is often not present even from patients diagnosed with fungal diseases, and invasive procedures require to obtain histological specimens (8, 27, 35, 38).

Galactomannan (GM) is a polysaccharide fungal cell wall component that is released during tissue invasion by *Aspergillus* hyphae and that can be detected in body fluids including BAL. GM detection in serum and BAL fluids as a means of establishing early diagnosis of IA in patients at risk is very promising. GM test shows a sensitivity that may range from 60% to 100% and specificity from 81% to 99% in neutropenic patients, depending of the tested sample (serum or BAL) and cut-off values (14, 36).

In Iran, there is no report on invasive fungal infections in ICU patients; however, some publications have focused on the *Aspergillus* as the causative agent of invasive infections in immunocompromised patients (2, 3). According to these facts, we assessed the ICU patients for IA with culture and non-culture based diagnostic methods from Iran.

Material and Methods

Patients

During one year, 818 admitted patients to our medical ICU were reviewed for inclusion in this prospective...
study. The study was approved by the ethics committee of Mazandaran University of Medical Sciences and written and informed consent was obtained from the patient or next of kin.

Patients who were older than 16 years and displayed at least one of the following host factors were enrolled in the study:
1. A hematologic malignancy, unless they were already treated with antifungals
2. Cancer and receiving chemotherapy within the last 3 months before admission
3. Neutropenia
4. Chronic obstructive pulmonary disease (COPD)
5. Solid organ transplant recipient
6. Steroid use: at least 4 mg methylprednisolone (or equivalent) a day for at least 7 days in the past 3 weeks before admission or during the course of the ICU stay for at least 5 days or a cumulative dose of at least 250 mg of methylprednisolone (or equivalent) in the past 3 months before enrollment
7. Recipient of any other immunosuppressive treatment (tacrolimus, cyclosporine, methotrexate, cyclophosphamide, sirolimus)
8. ICU stay more than 21 days

Laboratory work

Fiberoptic bronchoscopy (Olympus BF20D) with BAL (25–30 ml) was performed twice weekly thereafter if feasible. The sampling area was selected based on the infiltrate location on the chest radiograph. The presence of any tracheal or bronchial lesions was recorded by the endoscopist. A total of sixty eight BAL fluid samples were collected. BAL samples were not obtained from 5 patients due to their critically ill conditions or death, in second sampling time. The sampling area was selected based on the infiltrate location on the chest radiograph. The presence of any tracheal or bronchial lesions was recorded by the endoscopist. Out of 36 suspected patients to IA, 13 cases (36.1%) showed IA. According to criteria presented by EORTC/MSG, they were categorized as: 4 cases (30.8%) of possible IA and 9 (69.2%) of probable IA. Table 1 shows the findings in BAL samples as well as underlying conditions, type of IA and outcome of disease in ICU patients with MSG, they were categorized as: 4 cases (30.8%) of possible IA and 9 (69.2%) of probable IA. Table 1 shows the findings in BAL samples as well as underlying conditions, type of IA and outcome of disease in ICU patients with IA, 11(52.4%) of these samples were positive for septate hyphae and Aspergillus growth, respectively. Among Aspergillus species, A. flavus and A. fumigatus were equally isolated from positive samples for Aspergillus growth. Of 21 BAL samples, 16 (76.2%) were positive for GM. Other collected BAL samples had GM index ≥0.5 and <1.0. Of 21 BAL samples, 6 (28.6%) samples that were negative for Aspergillus growth on culture had GM index <1.0. From 13 patients with IA, 11(84.6%) had at least one positive BAL GM index. Of these patients, 9(81.8%) showed probable IA. Five patients (38.5%) were positive for GM in both sampling times. Three patients who were positive for GM in first BAL sample died before second sampling time. The main underlying predisposing conditions were neutropenia.
nia (53.8%), COPD (30.8%) and hematologic malignancy (15.4%). Of 13 patients with IA, Eight (61.5%) died. The mortality percentage among patients with probable and possible IA was 66.7% and 50%, respectively.

**Discussion**

In this present study, we presented a population of 818 ICU patients of which 4.4% had host factor, clinical or mycological criteria for infection with *Aspergillus*. This rate is in line with data of previous studies (24). Out of 36 cases of our ICU patients who met criteria for *Aspergillus* infection, 36.1% showed IA. The mortality percentage among IA patients was 61.5%. The previous studies have shown a diverse incidence of IA in ICU patients (10, 24, 37). Meersseman et al. (24) in a review paper presented an incidence of IA from 0.33% to 19% among ICU patients. Recently, Tortorano et al. (37) in a prospective survey in 38 ICUs of 27 Italian hospitals reported the median rate of 6.31 per 1000 admissions (range 1.26–28.64) for *Aspergillus* infection. This diversity may be due to difference in design of study, duration of study, type of underlying or immunosuppressive conditions and diagnosis methods. In addition, some authors suggested that invasive fungal infections are among the most commonly missed diagnoses in ICU patients (7, 28, 34).

In the present study we applied GM detection in BAL as one of the mycological criteria. However the Platelia *Aspergillus* GM EIA was designed to detect the presence of GM in serum samples but some authors suggested that it can be beneficial to detect GM in BAL fluid for IA diagnosis with high sensitivities and specificities (6, 20, 32). The previous studies (4, 19, 20) have shown that a GM index of ≥1.0 in comparison with 0.5 in BAL fluid enhances the diagnosis of IA in patients who are at risk; so in the present study, we used this GM index as a cutoff value for positive BAL GM.

The mortality percentage in our study was 66.7% and 50% among patients with probable and possible IA, respectively. These data are concordant with other previous studies (10–12, 24) however Meersseman et al. (25) reported a mortality rate for IA in ICU patients up to 97%. It should also be indicated that aspergillosis is more severe than candidiasis as the most prevalent fungal infections among ICU patients (37).

Eleven Of 21 BAL samples (52.4%) from IA patients were positive both for GM and for *Aspergillus* growth on culture. On the other hand, six samples (28.6%) that were

<table>
<thead>
<tr>
<th>Patient</th>
<th>Microscopic results</th>
<th>Culture results</th>
<th>BAL GM results</th>
<th>Underlying condition</th>
<th>IA</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First sample</td>
<td>Second sample</td>
<td>First sample</td>
<td>Second sample</td>
<td></td>
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</tr>
<tr>
<td>1</td>
<td>H</td>
<td>–</td>
<td>A. <em>fumigatus</em></td>
<td>–</td>
<td>3.3</td>
<td>Neutropenia</td>
</tr>
<tr>
<td>2</td>
<td>H</td>
<td>H</td>
<td>Neg</td>
<td>Neg</td>
<td>0.6</td>
<td>Neutropenia</td>
</tr>
<tr>
<td>3</td>
<td>H</td>
<td>H</td>
<td>A. <em>fumigatus</em></td>
<td>A. <em>fumigatus</em></td>
<td>3.2</td>
<td>COPD</td>
</tr>
<tr>
<td>4</td>
<td>H</td>
<td>H</td>
<td>Neg</td>
<td>Neg</td>
<td>0.6</td>
<td>Neutropenia</td>
</tr>
<tr>
<td>5</td>
<td>H</td>
<td>H</td>
<td>A. <em>fumigatus</em></td>
<td>A. <em>fumigatus</em></td>
<td>2.6</td>
<td>COPD</td>
</tr>
<tr>
<td>6</td>
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<td>H</td>
<td>Neg</td>
<td>Neg</td>
<td>0.7</td>
<td>COPD, Diabetes</td>
</tr>
<tr>
<td>7</td>
<td>H</td>
<td>–</td>
<td>A. <em>fumigatus</em></td>
<td>–</td>
<td>2.3</td>
<td>Hematologic malignancy</td>
</tr>
<tr>
<td>8</td>
<td>H</td>
<td>H</td>
<td>A. <em>fumigatus</em></td>
<td>A. <em>fumigatus</em></td>
<td>2.8</td>
<td>Neutropenia</td>
</tr>
<tr>
<td>9</td>
<td>H</td>
<td>H</td>
<td>Neg</td>
<td>Neg</td>
<td>2.8</td>
<td>COPD, ICU stay longer than 21 days</td>
</tr>
<tr>
<td>10</td>
<td>H</td>
<td>–</td>
<td>A. <em>fumigatus</em></td>
<td>–</td>
<td>3.2</td>
<td>Hematologic malignancy</td>
</tr>
<tr>
<td>11</td>
<td>H</td>
<td>H</td>
<td>A. <em>flavus</em></td>
<td>A. <em>flavus</em></td>
<td>3.3</td>
<td>Neutropenia</td>
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<tr>
<td>12</td>
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<td>–</td>
<td>Neg</td>
<td>–</td>
<td>2.9</td>
<td>Neutropenia</td>
</tr>
<tr>
<td>13</td>
<td>H</td>
<td>–</td>
<td>Neg</td>
<td>–</td>
<td>3.2</td>
<td>Neutropenia</td>
</tr>
</tbody>
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**Tab. 1:** Data for ICU patients with probable or possible aspergillosis included in this study (n = 13)
negative for *Aspergillus* growth on culture had GM index <1.0. This results show that the detection of GM in BAL fluid enhances the identification of *Aspergillus* species as a cause of pulmonary disease in ICU patients. The result of several studies have also shown that the GM analysis of BAL samples should consider for IA diagnosis among high-risk patients, especially for the ease of processing and the more rapid result than traditional methods (1, 23, 29). Moreover, all patients with probable IA had at least one positive BAL GM index, in the present study. This result can account for the importance of GM detection in the process of IA.

In the present study *A. fumigatus* and *A. flavus* were equally isolated from the BAL samples of patients with IA. In most previous studies, *A. fumigatus* was the predominant species of *Aspergillus* from patients with IA (10, 21, 33, 37). In our previous environmental studies *A. flavus* also was the most distributed species among genus *Aspergillus* in indoor and outdoor environment (16, 17). More distribution of *A. flavus* in environment can facilitate exposure and increase the risk of the colonization with this species.

In this present study the main underlying predisposing factors was neutropenia followed by COPD and hematologic malignancy. However neutropenia was reported as the main predisposing factor for IA but patients with COPD have emerged to be at particular risk for IA (10, 15, 25, 30). Because of long-term corticosteroid use by COPD patients, which has significant impact on the distribution and function of neutrophils, macrophages, and lymphocytes, COPD can be considered as one of the main predisposing factors for IA.

**Conclusion**

The results of our study showed that IA is a life threatening infectious disease in ICU. Our study has also indicated that non-classical risk factors especially COPD must be considered as a main predisposing condition for occurrence of aspergillosis in ICU patients. Our data have also revealed that GM detection in BAL samples play a significant role to IA diagnosis.

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**References**


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