Polymerase chain reaction (PCR) diagnosis and identification of mucormycosis in patients with suspected invasive fungal infection

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Background: Accurate diagnosis of mucormycosis, a life-threatening fungal infection, remains a challenge for physicians.

Objectives: To identify the causative Mucorales in fresh clinical samples and formalin-fixed paraffin-embedded (FFPE) samples of patients with proven mucormycosis by using semi-nested PCR of Mucorales targeting 18S ribosomal DNA and sequencing.

Patients/Methods: All patients with proven mucormycosis according to the EORTC/MSG criteria admitted between 2015 and 2017 and histopathologically proven FFPE samples were included. PCR targeting the 18S rDNA of Mucorales and ITS region was performed and PCR products were then sequenced.

Results: From 2015 to 2017, 63 patients suspected to have invasive fungal infection and meeting the inclusion criteria were enrolled in our study. Based on direct microscopic examination and culture of fresh clinical specimens, 9 (14.3%) cases of mucormycosis, 7 (11.1%) cases of aspergillosis, 4 (6.3%) cases of candidiasis and 1 (1.6%) case of cryptococcosis were diagnosed. Based on the EORTC/MSG criteria for invasive fungal diseases, 9 mucormycosis cases were included as proven. Five of 9 (55.6%) mucormycosis cases were confirmed by culture to be caused by *Rhizopus* species by standard phenotypic methods.

Overall, the species of Mucorales were *Rhizopus arrhizus* in 16 of 27 (59.3%) cases, *Rhizopus arrhizus* / *Amylomyces rouxii* in 2 of 27 (7.4%) cases and *Rhizopus stolonifer* in one of 27 (3.7%) cases (Fig. 1 and Table 1). The Mucorales and ITS PCR were negative in eight of 27 (29.6%) proven mucormycosis cases. Among these cases, 7 (25.9%) were male and 20 (74.1%) were female, the mean age of the patients was 52.6 ± 18.5 years (range 4-78). Of these, 17 (63.0%) patients died and 10 (37.0%) patients survived. Clinical presentations of the patients with mucormycosis classified into rhinocerebral (25; 92.6%) and disseminated (2; 7.4%). Disseminated form is defined as the involvement of two or more noncontiguous organ systems. The most important predisposing factors for mucormycosis were diabetes mellitus (20; 74.1%) and neutropenia in (17; 63.0%) (Table 2). The mortality rate after 2 months of follow-up is indeed very high (63.0%). All (5; 18.5%) patients with hematologic were died.

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Table 2. Underlying conditions predisposing to mucormycosis and mortality

<table>
<thead>
<tr>
<th>Underlying condition</th>
<th>Number of patients (%)</th>
<th>Number of patients (%) who died</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes mellitus</td>
<td>20 (74.1)</td>
<td>11 (55.0)</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>17 (60.0)</td>
<td>11 (64.7)</td>
</tr>
<tr>
<td>Use of immunosuppressive drugs</td>
<td>7 (25.9)</td>
<td>5 (71.4)</td>
</tr>
<tr>
<td>Hematologic malignancies</td>
<td>5 (18.5)</td>
<td>5 (100.0)</td>
</tr>
<tr>
<td>Organ transplant recipients</td>
<td>1 (3.7)</td>
<td>0 (0.0)</td>
</tr>
</tbody>
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