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

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Table 2. Underlying conditions predisposing to mucormycosis and mortality

Underlying condition	Number of patients (%)	Number of patients (%) who died
Diabetes mellitus	20 (74.1)	11 (55.0)
Neutropenia	17 (63.0)	11 (64.7)
Use of immunosuppressive drugs	7 (25.9)	5 (71.4)
Hematologic malignancies	5 (18.5)	5 (100.0)
Organ transplant recipients	1 (3.7)	0 (0.0)

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, 27 cases with histological evidence of mucormycosis were found in this study. PCR assays were performed on DNA extracted from 18 FFPE samples, 4 fresh tissues and 5 grown colonies (Fig.1). Amplification of DNA yielded a band of approximately 400 base pairs (bp) by external primers (ZM1 and ZM2) in 11 cases and a band of approximately 170 bp in gel electrophoresis by internal primers (ZM1 and ZM3) in 4 cases and by ITS primers in 4 cases.

The sequencing results for PCR product of 5 positive cultures were *Rhizopus arrhizus* (Fig. 1). PCR was also performed on 4 fresh tissues, in which direct microcopy exam was positive and culture did not grow. For these samples, sequencing results revealed *Rhizopus arrhizus* (Fig. 1). Among 18 samples of FFPE from the histopathologically proven cases of mucormycosis, 10 cases were *Mucorales* PCR positive. Among 9 fresh samples, 7 (77.8%) *Mucorales* PCR (5 in 1st and 2 in 2nd PCR run) and 2 (22.2%) ITS PCR were positive.

Figure 1: Mucormycosis diagnoses flow chart.

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.