Cutaneous mucormycosis and motor vehicle accidents: Findings from an Australian case series

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Abstract

Cutaneous disease is the third most frequent manifestation of mucormycosis. The clinical manifestations of and subsequent mortality due to cutaneous mucormycosis are dependent on the mode of acquisition and the host immune status. Here, we describe the epidemiology, clinical presentation, microbiology, and outcomes of 16 cutaneous mucormycosis infections managed in an Australian tertiary hospital over a 15-year period. The proportion with localized (56%), deep (38%), and disseminated (6%) cutaneous disease as well as the overall mortality (25%) were consistent with findings reported in the published literature. Two novel forms of hospital-acquired infection were reported following a sacral pressure sore and insertion of a foreign body during a bone graft procedure. The majority of patients were immunocompetent (75%) and/or suffered trauma (56%) with associated environmental contamination. A novel finding was that motor vehicle accidents (MVAs) accounted for 78% of all trauma-related cases, suggesting MVAs should receive greater recognition as a potential precipitant of cutaneous mucormycosis. Aggressive decontamination and debridement of devitalized tissue following trauma is therefore likely to play an important role in the prevention of this rare but potentially devastating infection.

Key words: mucormycosis, motor vehicle accident, Australia.

Introduction

Mucormycosis refers to infections caused by fungi of the phylum Zygomycota, subphylum Mucormycotina, order Mucorales [1]. It is the third most common form of invasive fungal infection, and its incidence is increasing [2]. Unlike other filamentous fungi, members of Mucorales can
more frequently cause disease in immunocompetent hosts [3], especially in the form of cutaneous disease. This is generally caused by trauma [3] in which disruption of the skin barrier allows implantation of fungal elements and subsequent vasotropic tissue invasion. Although mucormycosis is ubiquitous in nature, its epidemiology demonstrates significant geographic variations [4]. To date, descriptions of cutaneous mucormycosis from the Australian continent have been limited to case reports [5–8]. Our aim in this study was to describe the epidemiology of cutaneous mucormycosis in Western Australia and the associated clinical presentations, microbiology, and treatment outcomes.

Materials and methods
Study population and case definition
This study included patients managed between January 1997 and December 2012 at the Royal Perth Hospital, a 724-bed tertiary hospital in Perth, Western Australia. Cases were defined as those involving cutaneous tissue specimens that either yielded a Mucorales isolate in culture and/or a Mucorales that was identified in fungal microscopic/histopathologic studies and in which Mucorales was thought to be causing disease as assessed by an infectious diseases physician. Cutaneous mucormycosis was classified according to previously established criteria [3] as localized (limited to the cutaneous or subcutaneous tissue), deep (also involving muscle, fascia, bone, or tendon), or disseminated (involving cutaneous tissue and at least one noncontiguous site). The medical records of cases were retrospectively reviewed to obtain demographic, clinical (host factors and the type of underlying disease at the time of diagnosis of infection), microbiological, treatment, and outcome data. Study approval was obtained from the Royal Perth Hospital Human Research Ethics Committee (EC 2012/057).

Sampling, culturing, and strain identification
Skin biopsy (tissue) specimens were routinely Gram stained for microscopic studies and inoculated onto aerobic and anaerobic horse blood agar, chocolate agar, and cysteine lactose electrolyte-deficient agar. When fungal culture was specifically requested, the specimen was additionally stained using calcofluor white and potassium hydroxide for microscopic observations and cultured on Sabouraud glucose agar. A request for histopathology prompted additional staining using hematoxylin–eosin and periodic acid–Schiff procedures. All isolates were identified to the species level by examination of micro- and macromorphologic features in accordance with standard morphological criteria [9]. Molecular identification involved comparative sequencing of the internal transcribed spacer (ITS1–5.8S-ITS2 rRNA region sequence data of the isolated strains according to previously published criteria [10]. In two cases, direct polymerase chain reaction (PCR) and DNA sequencing of the ITS regions were performed on the tissue biopsy using a direct sequencing approach [11].

DNA extraction, PCR, sequencing, and analysis
Prior to 2006, DNA extraction was performed as described previously using a manual method [10]. All other isolates were extracted using an automated method in which the samples were extracted using the MagNA Pure LC robotic instrument (Roche, Castle Hill, NSW, Australia). Candida albicans (American Type Culture Collection 14053) was used as a positive control for DNA isolation, PCR, and DNA sequencing in each run. The oligonucleotide primers used for amplification and sequencing of the ITS regions were those described previously [10,11]. These primers bind to conserved regions of rRNA genes and amplify a product that encompasses a portion of the 18S and 26S rRNA gene and the entire intervening ITS1, 5.8S, and ITS2 rRNA gene regions. Sequence similarity searching was performed using the basic alignment search tool (BLAST). Sequence-based identification was performed using previously reported criteria [10,11].

Results
During the 15-year study period, members of Mucorales were detected in cutaneous tissue specimens from 21 patients, of which 16 were deemed to represent disease and therefore included in the case series. In 13 cases, mucormycosis was confirmed by both histopathology/fungal microscopy and culture, 2 by culture alone, and 1 by histopathology examination alone. Thus, 14 cases fulfilled the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group criteria for proven invasive fungal disease [12]. In two cases, the diagnosis of Mucorales infection was confirmed by direct PCR of material from tissue biopsies before an appropriate diagnosis could be obtained through fungal culture.

The demographics, presenting clinical manifestations, risk factors, microbiology, treatment regimes, and outcomes are outlined in Table 1. Seventy-five percent of the patients were male, with a median age of 45 years (range, 17–74 years). Twelve (75%) patients were immunocompetent, while 9 (56%) and 7 (44%) were associated with trauma and motor vehicle accidents (MVAs), respectively. Of those involving MVAs, four patients were unrestrained
<table>
<thead>
<tr>
<th>Case</th>
<th>Age/sex</th>
<th>Classification</th>
<th>Lesion</th>
<th>Genus/species</th>
<th>Motor vehicle accident</th>
<th>Immunosuppressed</th>
<th>Diabetes</th>
<th>Other risk factors</th>
<th>Surgery (no.)</th>
<th>Antifungal therapy</th>
<th>Adjuvant therapy</th>
<th>Outcome (if death, days post-onset of disease)</th>
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<tbody>
<tr>
<td>1</td>
<td>65/F</td>
<td>Localized</td>
<td>Necrotic wound</td>
<td>Rhizopus microsporus</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>9</td>
<td>L-amphoB (3), posaconazole (21)</td>
<td>No</td>
<td>Survived</td>
</tr>
<tr>
<td>2</td>
<td>72/M</td>
<td>Localized</td>
<td>Necrotic wound</td>
<td>Mucor circinelloides</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>2</td>
<td>C-amphoB (15)</td>
<td>None</td>
<td>No, Survived</td>
</tr>
<tr>
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<td>29/F</td>
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<td>Necrotic wound</td>
<td>Mucor circinelloides</td>
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<td>No</td>
<td>No</td>
<td>Dog bite</td>
<td>9</td>
<td>C-amphoB (15)</td>
<td>None</td>
<td>No, Survived</td>
</tr>
<tr>
<td>4</td>
<td>46/M</td>
<td>Localized</td>
<td>Necrotic wound</td>
<td>Rhizopus oryzae</td>
<td>No</td>
<td>Myeloma, autologous BMTx</td>
<td>Yes</td>
<td>Chemotherapy, steroids</td>
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<td>C-amphoB (15)</td>
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<td>No, Survived</td>
</tr>
<tr>
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<td>Necrotic wound</td>
<td>Apophysomyces elegans degans</td>
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<td>No</td>
<td>No</td>
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<td>No, Survived</td>
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<td>Rhizopus microsporus</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>8</td>
<td>L-amphoB (20)</td>
<td>No</td>
<td>No, Survived</td>
</tr>
<tr>
<td>7</td>
<td>45/M</td>
<td>Localized</td>
<td>Necrotic wound</td>
<td>Lichtheimia corymbifera</td>
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<td>No</td>
<td>No</td>
<td>Industrial trauma</td>
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<td>None</td>
<td>No</td>
<td>No, Survived</td>
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<tr>
<td>8</td>
<td>22/M</td>
<td>Localized</td>
<td>Sinus post surgical wound</td>
<td>Unknown</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Foreign body (bone wax) post surgery</td>
<td>1</td>
<td>None</td>
<td>No</td>
<td>No, Survived</td>
</tr>
<tr>
<td>9</td>
<td>40/M</td>
<td>Deep</td>
<td>Necrotic wound</td>
<td>Rhizopus microsporus</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>12</td>
<td>L-amphoB (181), posaconazole (346)</td>
<td>Caspofungin (181), desferasrox</td>
<td>Survived</td>
</tr>
<tr>
<td>10</td>
<td>29/M</td>
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<td>Necrotic wound</td>
<td>Rhizopus microsporus</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>12</td>
<td>L-amphoB (52)</td>
<td>No</td>
<td>No, Survived</td>
</tr>
<tr>
<td>11</td>
<td>31/M</td>
<td>Deep</td>
<td>Necrotic wound</td>
<td>Saksenaea vasiformis</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Burn</td>
<td>5</td>
<td>L-amphoB (56), posaconazole (90)</td>
<td>No</td>
<td>Survived</td>
</tr>
<tr>
<td>12</td>
<td>74/M</td>
<td>Deep</td>
<td>Spontaneous cellulitis</td>
<td>Cunninghamella berthotolaeae</td>
<td>No</td>
<td>Myeloma</td>
<td>No</td>
<td>Chemotherapy</td>
<td>2</td>
<td>C-amphoB (4), L-amphoB (90)</td>
<td>No</td>
<td>Survived</td>
</tr>
<tr>
<td>13</td>
<td>51/F</td>
<td>Localized</td>
<td>Mandibular papule</td>
<td>Rhizopus microsporus</td>
<td>No</td>
<td>Acute lymphoblastic leukemia, autologous BMTx</td>
<td>No</td>
<td>Steroids</td>
<td>1</td>
<td>L-amphoB (25)</td>
<td>No</td>
<td>Death (28)</td>
</tr>
</tbody>
</table>

Table 1. Epidemiology, clinical features, and treatment outcomes of patients with cutaneous mucormycosis.
Table 1. continued

<table>
<thead>
<tr>
<th>Case</th>
<th>Age/sex</th>
<th>Classification</th>
<th>Lesion</th>
<th>Genus/species</th>
<th>Motor vehicle accident</th>
<th>Immunosuppressed</th>
<th>Diabetes</th>
<th>Other risk factors</th>
<th>Surgery (no.)</th>
<th>Antifungal therapy (days)</th>
<th>Adjunctive therapy</th>
<th>Outcome (if death, days post-onset of disease)</th>
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</thead>
<tbody>
<tr>
<td>14</td>
<td>17/F</td>
<td>Deep</td>
<td>Necrotic wound</td>
<td><em>Apophysomyces variabilis</em></td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>8</td>
<td>L-amphoB (5)</td>
<td>No</td>
<td>Death (11)</td>
</tr>
<tr>
<td>15</td>
<td>73/M</td>
<td>Deep</td>
<td>Spontaneous necrotic ulcer</td>
<td><em>Rhizopus oryzae</em></td>
<td>No</td>
<td>Renal transplant</td>
<td>No</td>
<td>Steroids</td>
<td>2</td>
<td>L-amphoB (6)</td>
<td>No</td>
<td>Death (11)</td>
</tr>
<tr>
<td>16</td>
<td>59/M</td>
<td>Disseminated</td>
<td>Necrotizing fasciitis ankle and back</td>
<td><em>Apophysomyces elegans</em></td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>4</td>
<td>L-amphoB (3)</td>
<td>No</td>
<td>Death (3)</td>
</tr>
</tbody>
</table>

L-amphoB, liposomal amphotericin B; C-amphoB, conventional amphotericin B; BMTx, bone marrow transplant.

Discussion

Cutaneous mucormycosis accounts for 19% of all cases of mucormycosis [3], in which trauma, burns, surgery, surgical splints, arterial lines, injection sites, biopsy sites, tattoos, and insect or spider bites represent local risk factors [1]. Lesions are characterized by pain, erythema, and induration, with varying degrees of central necrosis similar to ecthyma gangrenosum. More advanced lesions take on the appearance of necrotizing fasciitis with gangrene and may rapidly lead to systemic dissemination [1]. Occasionally cutaneous disease is the initial manifestation of disseminated mucormycosis in immunocompromised patients, especially in the setting of neutropenia, leukemia, and immunosuppressive therapy [1]. In our case series, the proportions of patients with lesions that were localized (36%), deep (38%), and disseminated (6%) were consistent with the study by Roden and colleagues [3]. Systemic risk factors for cutaneous disease include trauma, burns, surgery, and immunosuppressive therapy, with the proportion of patients with trauma (one of whom had diabetes) with associated environmental contamination presenting with characteristic necrotic wounds.

A novel feature of our study was the high overall proportion of patients (seven of nine, 78%) whose trauma resulted from an MVA, which was significantly greater than the proportion (3% or 12%) previously described in the literature [1]. In our study, all nine immunocompetent patients who suffered trauma (one of whom had diabetes) with associated environmental contamination presented with characteristic necrotic wounds. A novel form of cutaneous disseminated disease was noted in one patient who developed disseminated cutaneous mucormycosis as a result of a nosocomial sacral pressure sore. None of the cases were associated with wound dressings, and none of the patients were receiving dextrometorphan, ketorolac, or intravenous immunoglobulin. The novel forms of hospital-acquired infection were noted in two novel forms of hospital-acquired infection, one of whom was a result of a nosocomial sacral pressure sore and the other three were motorcyclists. None of the cases were associated with wound dressings, and none of the patients were receiving dextrometorphan, ketorolac, or intravenous immunoglobulin.
Species causing cutaneous infection in *Rhizopus* Mucorales are ubiquitous; however, there were acquired from the northern part of the continent, which is in keeping with the known link between this genus and cutaneous infections in tropical and subtropical latitudes [19]. Although *Rhizopus* species predominated, the distribution of *Mucorales* species causing cutaneous infection in our case series differed from that described in published literature [15]. *Mucorales* are ubiquitous; however, there appears to be differences in their global ecology, the reasons for which remain unclear [4]. *Rhizopus*, *Mucor*, and *Apophysomyces elegans* have been recovered from environmental samples in Australia [1,7], although their relative distribution is unknown. To our knowledge, we are the first to report a case of *Apophysomyces variabilis* infection from Australia. All three of our cases that were attributable to *Apophysomyces* were acquired from the northern part of the continent, which is in keeping with the known link between this genus and cutaneous infections in tropical and subtropical latitudes [18]. *Apophysomyces* species have also been associated with the cutaneous form of mucormycosis [19]; however, we are unaware of any clear association between specific *Mucorales* species and MVAs.

Delayed diagnosis of mucormycosis is associated with poorer outcomes [11]. The median time from disease onset to diagnosis in our study (11.5 days) parallels that by other investigators (median, 1 week; range, 1–3 weeks) [20]. The highly variable clinical presentation of cutaneous mucormycosis makes early recognition difficult. However, there are diagnostic clues such as patients with a predisposing condition (as described above) or those who present with a rapidly progressive clinical course that is unresponsive to antibacterial therapy. A high index of suspicion is required; however, if a deep tissue biopsy is needed, it should be taken from the leading edge rather than the center of the cutaneous lesion [16]. The specimen should be sent for histopathology, fungal microscopy, and culture. The laboratory diagnosis of cutaneous mucormycosis can be difficult. Cultures for mucormycosis from superficial specimens are frequently negative [16]. Tissue biopsy should extend to subcutaneous fat, as hyphae that invade blood vessels of the dermis and subcutis are more likely to be visible and should be in sufficiently high concentration to be recoverable in culture [21]. In our case series, failure to request appropriate laboratory diagnostic techniques (histopathology and fungal culture) in a significant proportion of cases may have contributed to delays in diagnoses. Clinicians should specifically request cultures for *Mucorales* because routine homogenization of tissue in the laboratory can result in fungal destruction [1]. Hyphae may be seen with standard staining methods such as hematoxylin–eosin, periodic acid–Schiff, or Gomori methenamine silver [1]. The diagnosis is suggested by finding broad, infrequently septate hyaline hyphae. Vascular invasion with thrombosis and tissue necrosis is the pathological hallmark of mucormycosis [22]. *Mucorales* are typically fast growing, although identification of some species can be slow because of the need for special culture conditions [9]. Use of frozen sections has been proposed as another means of facilitating early diagnosis (6), although this has not proven to be successful when used routinely due to their low negative predictive value [23]. Lastly, we found that in some cases, direct PCR from tissues may permit rapid diagnosis of infection. However, more studies of this technique are needed.

The cornerstones of treatment have been early initiation of systemic antifungal therapy and repeated, aggressive surgical debridement of all necrotic tissue; however, this has not been examined through clinical trials. Serial frozen sections can be used effectively in some cases to evaluate surgical margins [24] as wound margins should be free of fungal hyphae after the final debridement. Basic principles of emergency wound care generally dictate that contaminated wounds be left open. However, in the setting of an outbreak following a tornado, immediate closure of traumatic wounds did not appear to be associated with cutaneous mucormycosis infection [25].

Compared with conventional amphotericin B, lipid formulations of amphotericin B are now the treatment of choice and should be administered intravenously at a minimum of 3–5 mg/kg/day [26]. Whether or not higher doses are associated with improved clinical outcomes is currently being explored in a phase 2 study [27]. The optimal duration of therapy is unclear [26], but the median duration of amphotericin B treatment in survivors in our study was 20 days (IQR, 14.5–75 days), suggesting that therapy should be prolonged. However, the development of acute renal impairment, as occurred in 7 of 13 (54%) patients in our study, may necessitate an earlier switch to oral
posaconazole [28], which is usually very well tolerated. However, wide variation in posaconazole serum drug concentrations [29] and species-specific minimum inhibitory concentrations [30] raise some concerns about its role in treatment of mucormycosis infections. The potential role for isavuconazole, a new broad-spectrum triazole, in the treatment of mucormycosis has yet to be determined. Minimum inhibitory concentrations are variable [31] and although approximately 45 patients with mucormycosis were enrolled in the VITAL trial, results are yet to be reported [32]. Hyperbaric oxygen has been reported as adjunctive treatment for mucormycosis, although the usefulness of this modality remains controversial [22]. Lastly, correction of underlying diseases such as ketoacidosis and reduction of immunosuppressive therapy, where possible, should be aggressively pursued [22].

Our overall mortality rate (25%) and significant proportion of patients with deep infection (Fig. 1) are reminders of the potential adverse consequences of this form of cutaneous infection. Consistent with other studies [33], we found deaths were associated with immunosuppressed host status and with having either deep or disseminated disease. In conclusion, our study demonstrates that MVAs may have been underestimated as a precipitant of cutaneous mucormycosis. We illustrated the complex relationships between host susceptibility and the risks, presentation, and outcome of cutaneous mucormycosis infections. Deep tissue specimens for histopathological and microbiological examination are required for an early diagnosis. Early surgical intervention with aggressive debridement of devitalized tissue and prompt initiation of antifungal therapy are essential for optimal outcomes.

Figure 1. Computed tomography scan reconstruction image of skull osteomyelitis secondary to cutaneous mucormycosis (case 11).
Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

References