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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 (ITS)1–5.8S-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 ITSl, 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 1. Epidemiology, clinical features, and treatment outcomes of patients with cutaneous mucormycosis.

Case	Age/sex	Classification	Lesion	Genus/species	Motor vehicle accident	Immunosuppressed	Diabetes	Other risk factors	Surgery (no.)	Antifungal therapy (days)	Adjuvant therapy	Outcome (if death, days post-onset of disease)
1	65/F	Localized	Necrotic wound	*Rhizopus microsporus*	Yes	No	No	No	9	L-amphoB (3), posaconazole (21)	No	Survived
2	72/M	Localized	Necrotic wound	*Mucor circinelloides*	Yes	No	No	No	2	None	No	Survived
3	29/F	Localized	Necrotic wound	*Mucor circinelloides*	No	No	No	Dog bite	9	C-amphoB (15)	No	Survived
4	46/M	Localized	Necrotic pressure sore	*Rhizopus oryzae*	No	Myeloma, autologous BMTx	Yes	Chemotherapy, steroids	None	C-amphoB (15)	No	Survived
5	23/M	Localized	Necrotic wound	*Apophysomyces elegans*	No	No	No	Insect bite	3	L-amphoB (14)	No	Survived
6	56/M	Localized	Necrotic wound	*Rhizopus microsporus*	Yes	No	No	No	8	L-amphoB (20)	No	Survived
7	45/M	Localized	Necrotic wound	*Lichtheimia corymbifera*	No	No	No	Industrial trauma	2	None	No	Survived
8	22/M	Localized	Sinus post surgical wound	*Unknown*	No	No	No	Foreign body (bone wax) post surgery	1	None	No	Survived
9	40/M	Deep	Necrotic wound	*Rhizopus microsporus*	Yes	No	No	No	12	L-amphoB (181), posaconazole (346)	Caspofungin (181), desferasirox	Survived
10	29/M	Deep	Necrotic wound	*Rhizopus microsporus*	Yes	No	No	No	12	L-amphoB (52)	No	Survived
11	31/M	Deep	Necrotic wound	*Saksenaea vasiformis*	Yes	No	No	Burn	5	L-amphoB (56), posaconazole (90)	No	Survived
12	74/M	Deep	Spontaneous cellulitis	*Cunninghamella bertholletiae*	No	Myeloma	No	Chemotherapy	2	C-amphoB (4), L-amphoB (90)	No	Survived
13	51/F	Localized	Mandibular papule	*Rhizopus microsporus*	No	Acute lymphoblastic leukemia, allogeneic BMTx	No	Steroids	1	L-amphoB (25)	No	Death (28)

Table 1. *continued*

Case	Age/sex	Classification	Lesion	Genus/species	Motor vehicle accident	Immunosuppressed	Diabetes	Other risk factors	Surgery (no.)	Antifungal therapy (days)	Adjuvant therapy	Outcome (if death, days post-onset of disease)
14	17/F	Deep	Necrotic wound	*Apophysomyces variabilis*	Yes	No	Yes	No	8	L-amphoB (5)	No	Death (11)
15	73/M	Deep	Spontaneous necrotic ulcer	*Rhizopus. oryzae*	No	Renal transplant	No	Steroids	2	L-amphoB (6)	No	Death (11)
16	50/M	Disseminated	Necrotizing fasciitis ankle and back	*Apophysomyces elegans*	No	No	Yes	No	4	L-amphoB (3)	No	Death (3)

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

passengers and the other three were motorcyclists. None of the cases were associated with wound dressings, and none of the patients suffered diabetic ketoacidosis, hemochromatosis, or were receiving desferrioxamine therapy. Two novel forms of hospital-acquired infection were noted, that is, case 4 acquired his infection as a result of a nosocomial sacral pressure sore and case 8 developed a cutaneous sinus 92 days following insertion of bone wax during a calvarial bone graft procedure.

The median time from the onset of symptoms to diagnosis was 11.5 days (interquartile range [IQR], 5.5–15 days). Direct microscopy was performed on all specimens and was positive for fungal elements in nine (56%) cases. At the time of diagnosis, a request for fungal culture or histopathological examination was made in only 6 (38%) and 11 (69%) cases, respectively. The median time to finding fungal growth in culture was 2 days (range, 1–3 days).

The mean daily doses of conventional amphotericin B and liposomal amphotericin B were 50 mg and 436 mg (range, 150–800 mg), respectively. Oral posaconazole 800 mg per day was used following amphotericin B therapy in three cases.

Discussion

Cutaneous disease 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 lesions produce aerial mycelia that are visible to the naked eye [1]. In our case series, the proportions of patients with localized (56%), deep (38%), and disseminated (6%) cutaneous disease was consistent with the finding by Roden in a larger case series [3]. Systemic risk factors for cutaneous mucormycosis include hyperglycemia, ketoacidosis, malignancy, leukopenia, and immunosuppressive therapy [1]. However, infection in immunocompetent hosts is well described, most typically after trauma, burns, or even cataclysmic events such as the tsunami in Southeast Asia [13]. 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 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 published literature [3,14,15]. Interestingly, all of our MVA-associated cases involved either unrestrained passengers or motorcycles, and this may have resulted in higher direct environmental trauma and, hence, greater opportunity for wound contamination with environmental *Mucorales*. As the statewide adult trauma service is based at our hospital, where road trauma is frequently seen, our findings might be partially explained by the fact that the cases were specifically referred to our facility. Nonetheless, we believe that MVAs should receive greater recognition as a potential precipitant of cutaneous mucormycosis. In this setting, suppression of host immune responses that is secondary to trauma and antibiotic use may also contribute to host susceptibility [16,17].

We observed a much wider spectrum of cutaneous manifestations in the five patients who did not experience trauma but instead had more traditional risk factors for mucormycosis including hematological malignancy (acute violaceous papule, spontaneous cellulitis, necrotic pressure sore), diabetes (disseminated necrotizing fasciitis), and solid organ transplant (spontaneous necrotic ulcer).

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

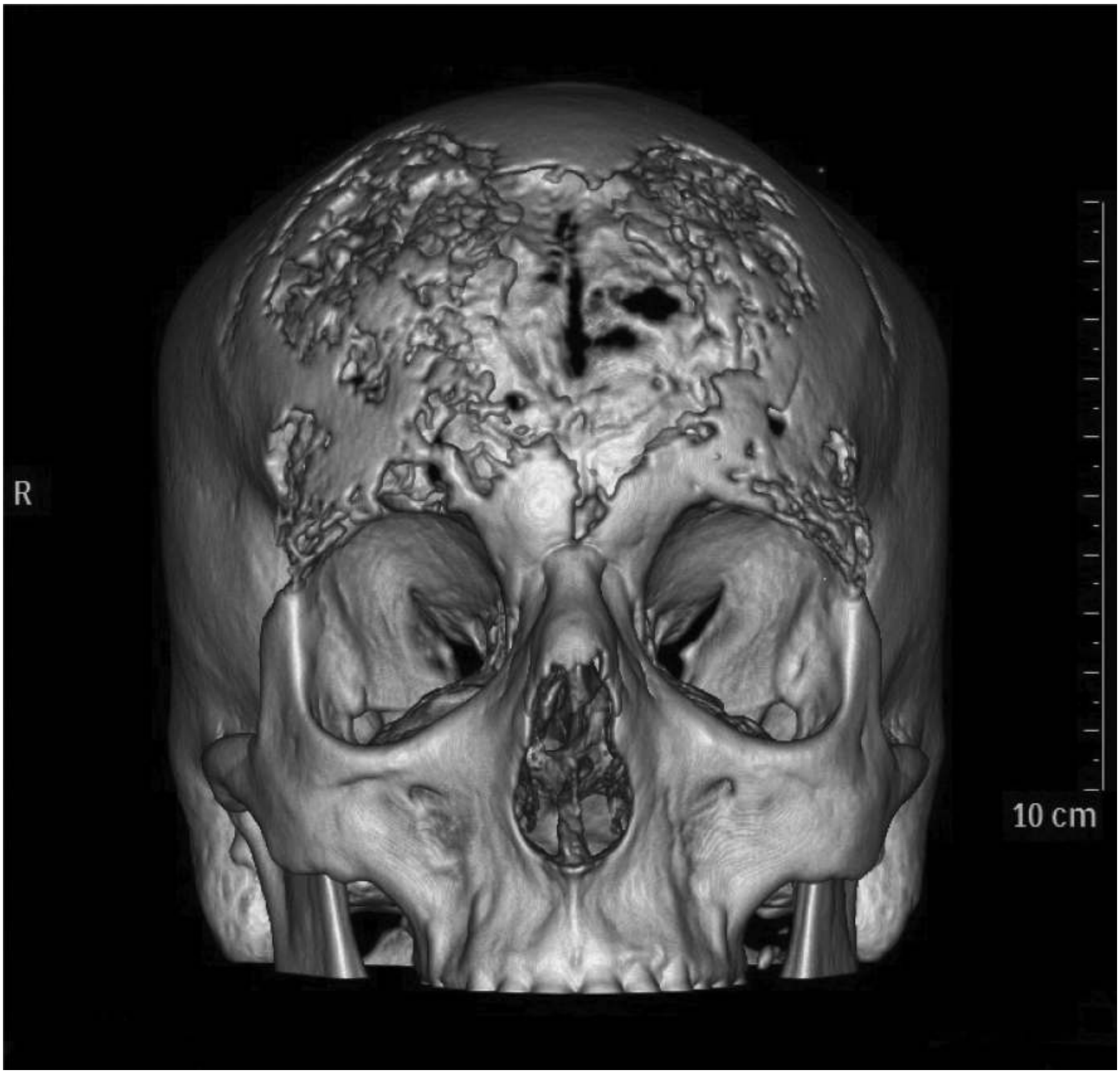

Figure 1. Computed tomography scan reconstruction image of skull osteomyelitis secondary to cutaneous mucormycosis (case 11).

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.

Declaration of interest

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

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