Clinical Microbiology and Infection 22 (2016) 775–781

Contents lists available at ScienceDirect

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.com

Original article

Mucormycosis in Australia: contemporary epidemiology and outcomes

K.J. Kennedy [1, *, †], K. Daveson [1, †], M.A. Slavin [2], S.J. van Hal [3], T.C. Sorrell [4], A. Lee [3], D.J. Marriott [5], B. Chapman [6, 7], C.L. Halliday [7], K. Hajkowicz [8], E. Athan [9], N. Bak [10], E. Cheong [11], C.H. Heath [12], C.O. Morrissey [13], S. Kidd [14], R. Beresford [15], C. Blyth [16], T.M. Korman [17], J.O. Robinson [12, 18], W. Meyer [6, 7], S.C.-A. Chen [7, 19], on behalf of the Australia and New Zealand Mycoses Interest Group of the Australasian Society for Infectious Diseases

[1] *Department of Infectious Diseases and Microbiology, Canberra Hospital, Australian National University Medical School, Canberra*
[2] *Department of Infectious Diseases, Peter MacCallum Cancer Centre, Victorian Infectious Diseases Service at the Doherty Institute for Infection and Immunity, Melbourne*
[3] *Departments of Infectious Diseases and Microbiology, Royal Prince Alfred Hospital, Sydney*
[4] *Centre for Infectious Diseases and Microbiology, Westmead Hospital and the Marie Bashir Institute for Infectious Diseases and Biosecurity, University of Sydney, Sydney*
[5] *Department of Microbiology and Infectious Diseases, St Vincent's Hospital, Sydney*
[6] *The Westmead Institute for Medical Research, The University of Sydney, Westmead, Sydney*
[7] *Centre for Infectious Diseases and Microbiology Laboratory Services, ICPMR, Westmead Hospital, Sydney*
[8] *Department of Infectious Diseases, Royal Brisbane and Women's Hospital, Brisbane*
[9] *Department of Infectious Diseases, Barwon Health, Deakin University, Geelong*
[10] *Department of Infectious Diseases, Royal Adelaide Hospital, Adelaide*
[11] *Department of Infectious Diseases and Microbiology, Concord Hospital, Sydney*
[12] *Department of Microbiology and Infectious Diseases, Royal Perth Hospital, School of Medicine and Pharmacology, University of Western Australia, Perth*
[13] *Department of Infectious Diseases, Alfred Health and Monash University, Melbourne*
[14] *National Mycology Reference Centre, SA Pathology, Adelaide*
[15] *Department of Infectious Diseases and Microbiology, Liverpool Hospital, Sydney*
[16] *School of Paediatrics and Child Health, University of Western Australia, Princess Margaret Hospital, Perth*
[17] *Monash Infectious Diseases and Monash University, Melbourne*
[18] *Australian Collaborating Centre for Enterococcus and Staphylococcus Species Typing and Research, School of Biomedical Sciences, Curtin University, School of Veterinary and Life Sciences, Murdoch University, Perth*
[19] *Centre for Infectious Diseases and Microbiology, The University of Sydney, Sydney, Australia*

ARTICLE INFO

Article history:
Received 25 October 2015
Received in revised form
30 December 2015
Accepted 9 January 2016
Available online 20 January 2016

Editor: E. Roilides

Keywords:
Apophysomyces
epidemiology
mortality
Mucorales

ABSTRACT

Mucormycosis is the second most common cause of invasive mould infection and causes disease in diverse hosts, including those who are immuno-competent. We conducted a multicentre retrospective study of proven and probable cases of mucormycosis diagnosed between 2004–2012 to determine the epidemiology and outcome determinants in Australia. Seventy-four cases were identified (63 proven, 11 probable). The majority (54.1%) were caused by *Rhizopus* spp. Patients who sustained trauma were more likely to have non-*Rhizopus* infections relative to patients without trauma (OR 9.0, p 0.001, 95% CI 2.1–42.8). Haematological malignancy (48.6%), chemotherapy (42.9%), corticosteroids (52.7%), diabetes mellitus (27%) and trauma (22.9%) were the most common co-morbidities or risk factors. Rheumatological/autoimmune disorders occurred in nine (12.1%) instances. Eight (10.8%) cases had no underlying co-morbidity and were more likely to have associated trauma (7/8; 87.5% versus 10/66; 15.2%; p <0.001). Disseminated infection was common (39.2%). *Apophysomyces* spp. and *Saksenaea* spp. caused infection in immuno-competent hosts, most frequently associated with trauma and affected sites other than lung and sinuses. The 180-day mortality was 56.7%. The strongest predictors of mortality were

* **Corresponding author**: K. Kennedy, Department of Infectious Diseases and Microbiology, Canberra Hospital & Health Services, PO Box 11, Woden ACT 2606, Australia
E-mail address: Karina.kennedy@act.gov.au (K.J. Kennedy).
† K.J. Kennedy and K. Daveson contributed equally.

http://dx.doi.org/10.1016/j.cmi.2016.01.005

mucormycete
mucormycosis
Rhizopus
Saksenaea
zygomycosis

rheumatological/autoimmune disorder (OR = 24.0, p 0.038 95% CI 1.2–481.4), haematological malignancy (OR = 7.7, p 0.001, 95% CI 2.3–25.2) and admission to intensive care unit (OR = 4.2, p 0.02, 95% CI 1.3–13.8). Most deaths occurred within one month. Thereafter we observed divergence in survival between the haematological and non-haematological populations (p 0.006). The mortality of mucormycosis remains particularly high in the immuno-compromised host. Underlying rheumatological/autoimmune disorders are a previously under-appreciated risk for infection and poor outcome. **K.J. Kennedy, CMI 2016;22:775**

Introduction

Infections caused by fungi of the order Mucorales are uncommon, but are significant for their aggressive nature, challenges in diagnosis and high mortality (40–100%) despite antifungal and surgical therapy [1–6]. The Mucorales are a diverse group of fungi associated with characteristic presentations according to underlying host conditions [1–3]. A significant proportion of infection also occurs in hosts with no apparent immune compromise, often in association with trauma [1–3,7,8].

The epidemiology of mucormycosis may be changing with the emergence of uncommon genera such as *Apophysomyces* [7,9], increasing numbers and breadth of immuno-compromised hosts [1] and outbreaks of infection following natural disasters and iatrogenic or other environmental exposure [7,10,11]. However, despite reports of rising incidence of mucormycosis [1,4,12], data on infection risks, treatment and outcomes are limited to case series and expert opinion. The epidemiology of mucormycosis varies between geographic regions [1–5,9,12], so knowledge of local patterns of disease is necessary to inform diagnosis and management. This multicentre study provides contemporary insights into the epidemiology, predisposing factors and determinants of outcomes of mucormycosis in Australia.

Materials and Methods

Study design

A retrospective multicentre, observational study of mucormycosis in adults was conducted across 15 Australian tertiary hospitals from 2004 to 2012 under the auspices of the Australia and New Zealand Mycoses Interest Group, and formed a substudy of a recently published analysis of invasive fungal disease (IFD) caused by non-*Aspergillus* moulds [13]. Institutional Human Research Ethics Committees approval was obtained at each site.

Collection of data

Cases were identified through a combination of pathology laboratory information systems, infectious diseases databases and hospital medical records coding [14]. A data review committee assessed each case for study inclusion. Only proven or probable infections were included. Patient demographics; co-morbid conditions (e.g. malignancy); predisposing factors for mucormycosis within the preceding 30 days of diagnosis (e.g. immunosuppressive therapies, trauma); site(s) of infection; microbiological and histological results; antifungal and other therapies; and clinical outcome at 30 and 180 days were collected.

Definitions

Proven or probable cases of mucormycosis were defined based on the European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) definitions for IFD [15]. However, in contrast to the EORTC/MSG criteria for assignment of probable IFD, host-specific criteria were not required.

Disseminated infection involved two or more non-contiguous sites or positive blood cultures. Gastrointestinal (GI) tissue infections included the intestinal tract, pancreas and liver. Prior antifungal use was defined as >7 days of treatment within the previous 30 days. Clinical outcome was assessed as all-cause mortality at 30 and 180 days.

Histopathological and microbiological examination

Clinical specimens were examined by experienced histopathologists at participating sites using local institutional protocols. The identity of cultured Mucorales was performed using standard phenotypic methods and, where available, characterized at a reference laboratory (Westmead Hospital or SA Pathology) by DNA sequencing targeting the internal transcribed spacer (ITS) regions or the D1/D2 regions of the 28S rRNA gene [16,17]. In some cases, panfungal PCR targeting the ITS 1 region was performed to identify Mucorales in histopathological specimens [18]. If species identification was not possible either by culture or by molecular methods, isolates were categorised either by genus or as 'unclassified Mucorales'.

Statistical analysis

Cases of 'unclassified Mucorales' were excluded when comparisons were made between genera. Categorical variables were compared using the χ^2 or Fishers exact test, and continuous variables by the Student's *t* test or Mann-Whitney *U* test where appropriate. Determinants of all-cause 30-day and 180-day mortality were examined by univariate and multivariate analysis. Backward, stepwise multivariate analysis was conducted with Firth's penalized-likelihood logistic regression due to the presence of complete separation of one variable. Variables with a univariate p value <0.20 were considered for inclusion in the model for multivariate analysis. Variables with p values ≤0.05 in a significant model with the largest penalized log likelihood were included within the final model. Kaplan–Meier survival analysis was performed for all-cause 180-day mortality and assessed by the log rank test. Calculations were computed using STATA (StataCorp. 2015. STATA Statistical Software: Release 14. College Station, TX).

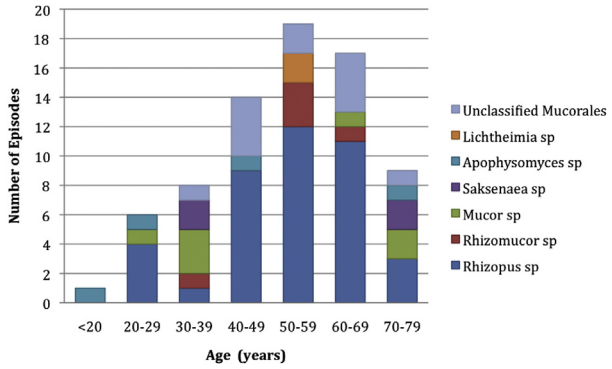

Fig. 1. Distribution of mucormycosis cases according to age and fungal genera.

Results

Seventy-four Mucorales infections were identified including 63 (85%) proven and 11 (15%) probable cases. There were no clusters of infection, with cases evenly distributed across the study period. The median patient age was 54 years (interquartile range (IQR) 42–63 years), and 48 (65%) of patients male. Although the number of cases varied across age groups, fungal aetiology was similar (Fig. 1). Thirty-three patients (45%) were admitted to an intensive care unit (ICU) at the time of diagnosis because of the fungal infection and/or underlying condition.

Diagnosis and aetiological agents

Seventeen cases (23%) of mucormycosis were diagnosed by histopathology without a positive culture. Twenty-five cases (34%) were diagnosed by culture without histopathology, and 32 cases (43%) were diagnosed by both culture and histopathology.

Molecular methods provided identification or confirmation of fungal identity to genus ($n = 9$) or species ($n = 26$) level in 35 (47%) cases (Table 1), including five cases diagnosed by histopathology without culture.

A broad range of pathogens were identified, with *Rhizopus* spp. accounting for the majority (Table 1). *Saksenaea vasiformis* complex and *Apophysomyces* species contributed over 10% of cases. One patient had infection with *Mucor circinelloides* (lung) and *Fusarium* spp. (blood and skin) resulting in death. No other mixed infections were identified.

Table 1
Pathogens causing mucormycosis: Australia 2004–2012

Genus		No. of isolates	No. identified by DNA sequencing[b]
Apophysomyces spp.		4 (5.4%)	
	Apophysomyces elegans	3	2
	Apophysomyces variabilis	1	1
Lichtheimia corymbifera		2 (2.7%)	2
Mucor spp.		7 (9.5%)	
	Mucor circinelloides	1	1
	Mucor indicus	1	1
	Mucor spp.	5	3
Rhizomucor spp.		5 (6.8%)	
	Rhizomucor miehei	1	1
	Rhizomucor pusillus	1	1
	Rhizomucor spp.	3	2
Rhizopus spp.		40 (54.1%)	
	Rhizopus microsporus	12	7
	Rhizopus arrhizus	10	4
	Rhizopus schipperae	1	1
	Rhizopus stolonifer	2	2
	Rhizopus spp.	15	4
Saksenaea vasiformis complex		4 (5.4%)	3
Unclassified Mucorales[a]		12 (16.2%)	
Total		74	35

[a] Characteristic hyphae of Mucorales seen on histopathology without identification by culture or DNA sequencing.
[b] DNA sequencing either (i) corroborated the species identification provided by phenotypic methods or (ii) provided genus-level and/or species-level identification where morphological methods failed to provide an identification.

Co-morbidity and predisposing factors

The commonest co-morbidities were haematological malignancy (48.6%) and diabetes mellitus (27%), although; none with ketoacidosis (Table 2). Rheumatological/autoimmune diseases were present in 9 (12.1%) cases (rheumatoid arthritis (n = 2), systemic lupus erythematosus (3), vasculitis (2), autoimmune hepatitis (1) and autoimmune haemolytic anaemia (1)). All but one of these patients had received prior treatment with corticosteroids, with combinations of other immunosuppressive drugs, including rituximab ($n = 1$), infliximab (1), calcineurin inhibitor (1) and other chemotherapeutic agents (3). Seventeen patients (22.9%) had antecedent trauma, including motor vehicle accidents and burns, with one patient sustaining massive injuries from the Boxing Day tsunami in Asia. Eight patients (10.8%) had neither underlying co-morbidity nor immuno-compromise, and were significantly more likely to have acquired infection through trauma (7/8; 87.5%) compared to trauma-related infections in those with an underlying co-morbidity and/or immune-compromise (10/66; 15.2%; p<0.001).

Patients who sustained trauma were significantly more likely to have non-*Rhizopus* infections compared with patients without trauma (11/15, 73.3% versus 11/47, 23.4%; OR 9.0; 95% CI 2.1–42.8; p 0.001).

Site of infection

The lung was the most common site of infection (44.6%), followed by sinuses (29.7%), skin and soft tissue (20.3%), brain (14.9%) and bone/joint (14.9%) (Table 3). Twenty-nine (39.2%) cases were disseminated, with individual sites represented in various combinations, including the lung ($n = 16$), sinus (10), brain (9) and skin (9). One patient had 4 sites and 6 patients 3 sites involved. All episodes of GI mucormycosis were disseminated. Of the patients with rheumatological/autoimmune diseases, the lung ($n = 5$), sinus (4), brain (3), eye (1), skin (1) and heart (1) represented the sites of infection, including 3 cases of disseminated disease.

Patient management

Antifungal treatment for mucormycosis was initiated in 64 patients (86.5%) and continued for a median of 64.5 days (IQR 21–365 days). Of those who survived 180 days ($n = 33$), the median treatment duration was 196 days (IQR 64–587 days).

Eight patients either died before diagnosis or were actively palliated without receiving antifungal therapy. Another patient received voriconazole alone and died of disseminated infection at 17 days. One patient had trauma-related soft-tissue infection, without underlying co-morbidity, was treated with surgery alone and was alive at 180 days.

An amphotericin B formulation (predominantly liposomal amphotericin B) was the mainstay of antifungal therapy (62/64

Table 2
Genera or groups of Mucorales by patient co-morbidity or predisposing factor[a]

Variable	All episodes (*n*=74)	Unclassified mucormycete (*n*=12)	*Rhizopus* spp. (*n*=40)	*Mucor* spp. (*n*=7)	*Rhizomucor* spp. (*n*=5)	*Saksenaea* spp. (*n*=4)	*Apophysomyces* spp. (*n*=4)	*Lichtheimia* spp. (*n*=2)
Co-morbidity								
None[b]	8 (10.8)	0	2[c] (5)	1 (14.3)	1 (20)	2 (50)	2 (50)	0
Diabetes mellitus	20 (27.0)	4 (33.3)	13 (32.5)	0	0	1 (25)	2 (50)	0
Chronic lung disease	11 (14.9)	4 (33.3)	3 (7.5)	1 (14.3)	1 (20)	1 (25)	0	1 (50)
Haematological malignancy[d]	36 (48.6)	5 (41.7)	20 (50)	6 (89.6)	4 (80)	0	0	1 (50)
Solid organ cancer	2 (2.7)	1 (8.3)	0	1 (14.3)	0	0	0	0
Bone marrow transplant	13 (17.6)	0	9 (22.5)	4 (57.1)	0	0	0	0
Solid organ transplant	8 (10.8)	1 (8.3)	6 (15)	0	0	0	0	1 (50)
Rheumatological / autoimmune conditions	9 (12.1)	2 (16.7)	4 (10)	1 (14.3)	2 (40)	0	0	0
Chronic renal failure	7 (9.5)	2 (16.7)	5 (12.5)	5 (71.4)	0	0	0	0
Predisposing factor								
Neutropenia	32 (43.2)	4 (33.3)	17 (42.5)	6 (85.7)	4 (80)	0	0	1 (50)
Corticosteroid use	39 (52.7)	3 (25)	25 (62.5)	6 (85.7)	4 (80)	0	0	1 (50)
Cancer chemotherapy	31 (41.9)	4 (33.3)	18 (45)	5 (71.4)	3 (60)	0	0	1 (50)
Calcineurin inhibitors	15 (20.2)	0	14 (35)	1 (14.3)	0	0	0	0
Monoclonal antibodies	7 (9.5)	2 (16.7)	4 (10)	1 (14.3)	0	0	0	0
Major or minor trauma	17 (22.9)	2 (16.7)	4 (10)	3 (42.9)	2 (40)	3 (75)	3 (75)	0
Major surgery	18 (24.3)	2 (16.7)	9 (22.5)	0	1 (20)	1 (25)	3 (75)	2 (100)
Hospital construction	10 (13.5)	3 (25)	4 (10)	1 (14.3)	2 (40)	0	0	0
Antifungal prophylaxis Medication[e,f]								
Any agent	34 (44.9)	5 (41.7)	19 (45.7)	6 (85.7)	3 (60)	0	0	1 (50)
Voriconazole	10 (13.5)	1 (8.3)	6 (15)	1 (14.3)	2 (40)	0	0	0
Posaconazole	10 (13.5)	2 (16.7)	6 (15)	2 (28.6)	0	0	0	0

[a] Expressed as number of patients (percentage of patients with co-morbidity or predisposing factor).
[b] Also no host immuno-compromise.
[c] *Rhizopus microsporus* ($n = 1$); *Rhizopus schippelae* ($n = 1$).
[d] Haematological cancers included: acute leukaemia ($n = 16$), chronic leukaemia ($n = 7$), lymphoma ($n = 5$), multiple myeloma ($n = 3$), myelodysplastic syndrome ($n = 4$) and other cancers ($n = 2$).
[e] Antifungal medications prescribed for at least 7 days within the preceding 30 days.
[f] Includes itraconazole (3), liposomal amphotericin B (1) and fluconazole (10).

Table 3
Mucormycosis: site of infection and 180-day mortality according to pathogen group[a]

Body site (total)	Unclassified Mucorales (*n*=12)	*Rhizopus* spp. (*n*=40)	*Mucor* spp. (*n*=7)	*Rhizomucor* spp. (*n*=5)	*Apophysomyces* spp. (*n*=4)	*Saksanaea vasiformis* complex (*n*=4)	*Lichtheimia corymbifera* (*n*=2)	180-day mortality, *n* (%)
Lung (*n*=33)	4	20	5	3	0	0	1	24 (72.7%)
Sinus (*n*=22)	5	13	2	1	0	0	1	14 (63.6%)
Skin/deep soft tissue (*n*=15)	3	5	1	2	2	2	0	7 (46.7%)
Brain (*n*=11)	1	5	0	1	1	1	2	9 (81.8%)
Bone/joint (*n*=11)	2	5	0	0	2	2	0	3 (27.3%)
Gastrointestinal tissue (*n*=7)	1	1	3	0	1	0	1	5 (71.4%)
Eye (*n*=4)	2	1	0	0	0	1	0	1 (25%)
Blood (*n*=2)	0	0	2	0	0	0	0	1 (50%)
Other (*n*=7)[b]	0	4	1	1	0	0	1	4 (57.1%)
Disseminated (*n*=29)	5	12	5	1	3	1	2	18 (62.1%)
180-day mortality (*n*=42; 57%)	5 (41.7%)	23 (57.5%)	6 (85.7%)	3 (60%)	2 (50%)	1 (25%)	2 (100%)	

[a] Numbers total more than 74 as many patients had more than one site of infection.
[b] Includes kidney ($n = 3$); endocardium ($n = 1$); gingival tissue ($n = 1$); genitalia ($n = 1$).

cases), with amphotericin B-based combination therapy administered in 11 cases (17.7%), including caspofungin ($n = 5$), posaconazole ($n = 5$) and terbinafine ($n = 1$). Posaconazole alone, or in combination with caspofungin, was used as definitive therapy in two patients. Posaconazole solution was used as step-down therapy in all cases where the patient was alive following initial 'induction antifungal therapy'. Adjunctive granulocyte–macrophage colony-stimulating factor or granulocyte infusion therapies were administered to two acute myeloid leukaemia patients with fatal lung (+/− sinus) infection. Surgery was performed in 44 of 64 (69%) patients receiving anti-fungal therapy.

Outcomes

The all-cause 30- and 180-day mortality was 42% ($n = 31$) and 56.7% [42] respectively. Deaths occurred at a median of 14 days (IQR 4–32 days, range 1–169 days) following diagnosis.

Table 4 demonstrates the variables associated with mortality at 180 days by univariate and multivariate analyses. Independent predictors of mortality on multivariate analysis were the presence of a rheumatological/autoimmune disorder (OR = 24.0, p 0.038, 95% CI 1.2–481.4), haematological malignancy (OR = 7.7, p 0.001, 95% CI 2.3–25.2) and ICU admission (OR = 4.2, p 0.02, 95% CI 1.3–13.8). The Kaplan–Meier survival curves for the whole patient

Table 4
Mucormycosis: univariate and multivariate analysis of overall 180-day mortality

Variable	Univariate analysis				Multivariate analysis	
	Died (*n*=42)	Alive (*n*=32)	p value	OR (95% CI)	p value	OR (95% CI)
Male gender (*n*=48)	26 (62%)	22 (69%)	0.54	–	–	–
Co-morbidities / Predisposing conditions						
Diabetes (*n*=20)	10 (24%)	10 (31%)	0.41	–	–	–
Chronic lung disease (*n*=11)	6 (14%)	5 (16%)	0.87	–	–	–
Chronic renal failure (*n*=7)	3 (7%)	4 (13%)	0.46	–	–	–
Rheumatological/ autoimmune disorders (*n*=9)	9 (22%)	0 (0%)	0.004	–[a]	0.038	24.0 (1.2–481.4) [a]
Haematological malignancy (*n*=36)	27 (64%)	9 (28%)	0.002	4.6 (1.7–12.3)	0.001	7.7 (2.3–25.2)
Bone marrow transplant (*n*=13)	10 (24%)	3 (9%)	0.132	–	–	–
Solid organ transplant (*n*=8)	4 (10%)	4 (13%)	0.69	–	–	–
No co-morbidities (*n*=8)	1 (2%)	7 (22%)	0.02	0.087 (0–0.59)	–	NS
Additional predisposing factors within the preceding 30 days						
Major/minor trauma (*n*=17)	7 (17%)	10 (31%)	0.11[b]	–	–	NS
Surgery (*n*=18)	10 (24%)	8 (25%)	0.9	–	–	–
ICU admission (*n*=33)	23 (55%)	10 (31%)	0.044	8.1 (2.9–23)	0.020	4.2 (1.3–13.8)
Hospital construction (*n*=10)	4 (10%)	6 (19%)	0.31	–	–	–
Cancer chemotherapy (*n*=31)	23 (55%)	8 (25%)	0.01	7.67 (2.56–23)	–	NS
Corticosteroids (*n*=39)	29 (69%)	10 (31%)	0.001	4.9 (1.8–13.1)	–	NS
Monoclonal antibodies (*n*=7)	6 (14%)	1 (3%)	0.13[b]	–	–	NS
Calcineurin inhibitors (*n*=15)	8 (19%)	7 (22%)	0.77	–	–	–
Neutropenia (*n*=32)	25 (60%)	7 (22%)	0.001	5.25 (1.89–14.6)	–	NS
Manifestations of infection						
Disseminated infection (*n*=29)	18 (43%)	11 (34%)	0.46	–	–	–
Antifungal treatments						
Surgery (*n*=45)	20 (51%)	25 (78%)	0.02	0.29 (0.11–0.83)	–	NS
Amphotericin B combination antifungal therapy (*n*=11)	6 (14%)	5 (16%)	0.87	–	–	–
Combination surgery and antifungal agent (*n*=44)	20 (48%)	24 (75%)	0.02	0.25 (0.09–0.71)	–	NS

[a] Rheumatological/autoimmune disorders is a perfect predictor of mortality and therefore OR, 95% CI and p values cannot be determined in the univariate analysis. Firth's logistic regression was used for multivariate calculations.
[b] Variables that were included in the multivariate analysis and remained in the final model but where p >0.05 on univariate analysis.

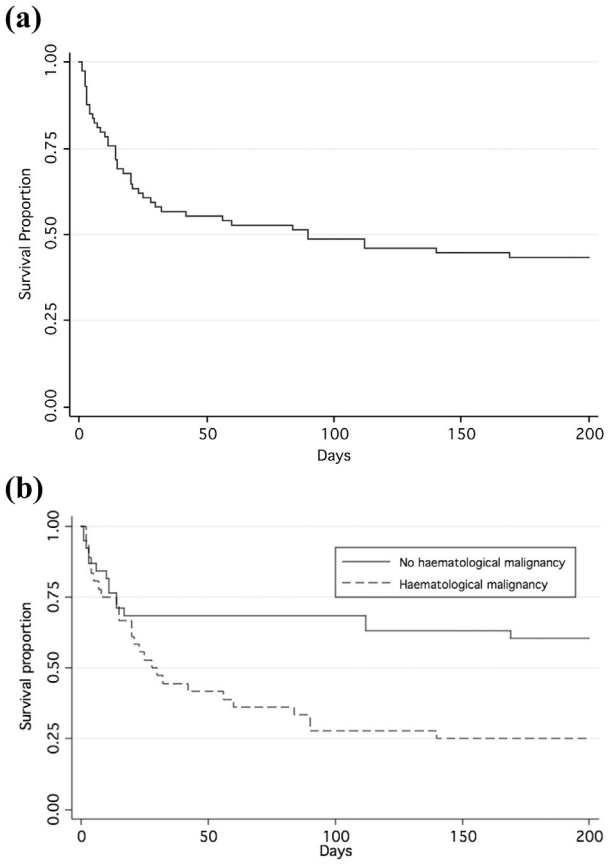

Fig. 2. (a) Kaplan–Meier survival curve of all patients with mucormycosis. (b) Kaplan–Meier survival curve stratified by the presence or absence of haematological malignancy (p 0.006).

cohort (Fig. 2a) and stratified by the presence of haematological malignancy (Fig. 2b), demonstrate that most deaths occurred within the first month, with subsequent divergence in survival between the haematological and non-haematological populations (p 0.006). There was no association between specific fungal genera and mortality (Table 3).

Discussion

Despite recent reports of increasing incidence [1,4,12], infections due to Mucorales remain infrequent. This multicentre study describes the epidemiology of mucormycosis across Australia, demonstrating the diversity in causative fungi and predisposing factors, and the overall high mortality despite intensive treatment that included surgical debridement in most cases. Underlying rheumatological/autoimmune disorders, haematological malignancy and ICU admission were variables associated with increased mortality.

Rhizopus spp. account for most cases of invasive mucormycosis, with *R. arrhizus* (formerly *R. oryzae*) the predominant species [1–3,5–7,19]. In this study, *R. microsporus* was most frequently isolated although almost 40% of *Rhizopus* spp. were not assigned to species level and hence certain species may have been underrepresented. *Rhizopus microsporus* has been associated with nosocomial outbreaks [11]; however, we did not observe any case clusters. Contrary to European studies where *Lichtheimia* spp. have represented up to one-third of cases [2,3,6,20], this genus was encountered infrequently. Of note, *Apophysomyces* spp. and *Saksenaea vasiformis* complex together caused 10.8% of cases compared with <5% in Europe [2,3,6,20]. In India however, *Apophysomyces* spp. is the second most common causative pathogen [12].

Both *Apophysomyces* and *Saksenaea* are soil saprophytes, with IFD occurring mainly in tropical and subtropical climates [21]. As noted by others [21], our eight patients were all

immunocompetent, had predominantly acquired infection through trauma (75%) and had infection frequently localized to the skin/soft tissue and bone/joint (50%). No infection involved the lung.

Excluding *Apophysomyces* and *Saksenaea* infections, the majority of hosts were immunocompromised, most commonly haematological malignancy and corticosteroid use. Indeed haematological malignancy has surpassed diabetes mellitus as the commonest predisposing factor in many regions [1–3,5] excluding countries, such as India [12]. Although diabetes was present in 27% of our patients, ketoacidosis was not observed [1].

A key finding from this study was the relatively high proportion of patients with rheumatological/autoimmune conditions (12.1% of cases) compared with previous studies (<2%) [1,2,22]. Such patients may be at risk for IFD because of intensive immune suppression, but have delayed diagnosis and institution of appropriate treatment due to under-recognition of the risk. Importantly, outcomes were particularly poor (100% mortality), indicating the need for heightened awareness among clinicians of mucormycosis and further research into risk profiles in this patient cohort.

Trauma, burns or surgery have been identified as risk factors for mucormycosis in as many as 19% of cases [1–3,9,23] and typically in immuno-competent hosts. Of the 17 trauma-associated cases in this series, 9 (53%) had disseminated mucormycosis, which may have contributed to the higher mortality (41%) than generally observed (22.9%) [8]. As observed previously [9], mucormycosis resulting from trauma was more commonly associated with non-*Rhizopus* Mucorales (73.3%).

Improvements in laboratory techniques over time have resulted in increases in the isolation rate of pathogenic Mucorales [1]. In this series, 77% of cases were diagnosed by culture with/without histopathology. Although DNA sequencing of the cultured isolate or directly from tissue (either fresh or retrieved specifically for this study), provided species or genus identification in 35 episodes, including five episodes diagnosed by histopathology alone, 9 Mucorales could not be assigned to a species. We used ITS sequencing as a first-line method for species identification as is recommended [24,25]. However, assays which target the ITS region lack sufficient discriminatory power, particularly if direct detection in tissue is attempted. Sequencing of alternate loci, such as the 18S and 28S rRNA regions and cytochrome b may be additionally required to speciate Mucorales [25]. Nonetheless, routine use of molecular diagnostics has the potential to improve the identification of Mucorales, particularly species such as *Apophysomyces* and *Saksenaea* spp. that do not sporulate well.

Infection sites were similar to previous reports [1–5,19] excluding higher occurrences of disseminated infection (39.2% vs 6.8–26%). All infections involving gastrointestinal tissue (9.5%) were disseminated. Gastrointestinal mucormycosis is uncommon and is predominantly associated with low-birthweight infants, malnutrition and peritoneal dialysis [1]. These predisposing factors were not noted in our study. Both patients with fungaemia had *Mucor* spp. infections, with one surviving beyond 6 months [26]. Positive ante-mortem blood cultures in the context of mucormycosis are extremely rare [27].

The 180-day mortality remains high (57%) despite antifungal therapy with or without surgery, and is comparable to that reported previously (44–59%) [1–3,5]. Despite the availability of amphotericin in the 1960s, there has been no significant improvement in mucormycosis-associated mortality [1]. However, over this time there has been an increase in the proportion of cases of mucormycosis in severely immuno-compromised individuals. Indeed, in our study, the poorest outcomes were identified for the first time in patients with rheumatological/autoimmune conditions, with independent predictors of death also including underlying haematological malignancy and ICU admission. Conversely, survival was greatest in those without co-morbidities. Divergence in survival between cases with and without haematological malignancy occurred approximately 1 month after diagnosis, suggesting that persisting immuno-compromise is the key to poor outcomes. This finding is important for the design of new clinical trials, as assessment of clinical endpoints may need to be undertaken at specific times in different populations.

Limitations of this study include its retrospective nature, which may explain the relatively low numbers of probable episodes, and trauma-related cases could be over-represented because tissue-confirmed cases are easier to acquire. Potential differences in clinical and diagnostic practice may have influenced case detection and outcomes. In particular, as there was no systematic use of molecular diagnostics, the diversity of Mucorales may have been underestimated, affecting the reported relationship between specific genera and clinical features and outcomes. The study did not capture all hospitals across Australia, hence the incidence of infection cannot be accurately determined.

In conclusion, this study provides insights into mucormycosis within Australia, and highlights the need for further research to facilitate risk stratification, case definitions in immuno-competent hosts, early diagnosis and optimal management to improve the currently poor outcomes.

Funding

The authors thank Pfizer Australia, Gilead Sciences Inc. and MSD Australia for their financial support of this study.

Acknowledgements

We thank hospital scientists at the participating institutions for their assistance in fungal identification, and Dr Anna Riddell, based at the Royal Darwin Hospital when the study was performed, and Claire Mahoney and Nicholas Cornish of Deakin University, for assistance in data collection. TCS is a Sydney Medical School Foundation Fellow whose work is funded by the National Health and Medical Research Council of Australia (#1050106).

The results of this study were presented by Monica Slavin at the 19th ISHAM Congress in Melbourne, Australia, 4–8 May 2015.

ANZMIG Business Committee Members

Queensland: Julia Clark, Royal Children's Hospital, Brisbane; Joseph McCormack, Mater Hospital, Brisbane; David Looke, Princess Alexandra Hospital. Brisbane; E. Geoffrey Playford, Princess Alexandra Hospital, Brisbane

New South Wales: Sharon Chen, Westmead Hospital, Sydney; Thomas Gottlieb, Concord Hospital, Sydney; Catriona Halliday, Westmead Hospital, Sydney; Deborah Marriott, St. Vincent's Hospital, Sydney; Brendan McMullan, Sydney Children's Hospital, Sydney; Wieland Meyer, Westmead Hospital, Sydney; Tania Sorrell, Westmead Hospital, Sydney, Sebastian van Hal, Royal Prince Alfred Hospital, Sydney

Victoria: Michelle Ananda-Rajah, The Alfred Hospital, Melbourne; C. Orla Morrissey, The Alfred Hospital, Melbourne, Monica Slavin, Peter MacCallum Cancer Institute, Melbourne.

South Australia: Narin Bak, Royal Adelaide Hospital, Adelaide; Sarah Kidd, SA Pathology, Adelaide

Western Australia: Ian Arthur, Sir Charles Gairdner Hospital, Perth; Christopher Blyth, Princess Margaret Hospital, Perth; Christopher Heath, Royal Perth Hospital, Perth

ACT: Karina Kennedy, Canberra Hospital, Canberra; Kathryn Daveson, Canberra Hospital, Canberra

New Zealand: Arthur Morris, Auckland Hospital, New Zealand; Steve Chambers, Christchurch Hospital, New Zealand

Transparency Declaration

See the Funding section for details.

Drug Advisory Boards

Sharon Chen, Monica Slavin, Sebastian van Hal, Christopher Heath, Deborah Marriott, C. Orla Morrissey and Tania Sorrell, are on the Antifungal Advisory Boards of Gilead Sciences Inc., MSD Australia and Pfizer Australia. Sarah Kidd is on the Antifungal Advisory Board of Pfizer Australia, MSD Australia and Mayne Pharma. Tony Korman is on the Antibacterial Advisory Board of Pfizer Australia.

Untied Educational Grants

Tania Sorrell, Deborah Marriott, Monica Slavin, Sharon Chen, Sebastian van Hal, Christopher Heath, Narin Bak, C. Orla Morrissey, Sarah Kidd and Wieland Meyer have received funding in the form of untied grants from Gilead Sciences Inc., MSD Australia and Pfizer Australia. Catriona Halliday and Sarah Kidd have received funding in the form of untied educational grants from Merck.

Tony Korman has received monetary reimbursement for service on speakers bureaus for Novartis (2010) and Merck (2011). Sharon Chen has received monetary reimbursement for service on speakers bureaus from Gilead Sciences Inc. (2014). Sarah Kidd has received monetary reimbursement for service on speakers bureaus from Gilead Sciences Inc. (2013). Monetary reimbursements on behalf of C. Orla Morrissey provided by her on speakers bureaus have been paid to Alfred Health from MSD Australia (2013), Pfizer Australia (2012) and Gilead Sciences Inc. (2012, 2013, 2014).

References

[1] Roden MM, Zaoutis TE, Buchanan WL, Knudsen TA, Sarkisova TA, Schaufele RL, et al. Epidemiology and outcome of zygomycosis: a review of 929 reported cases. Clin Infect Dis 2005;41:634–53.
[2] Lanternier F, Dannaoui E, Morizot G, Elie C, Garcia-Hermoso D, Huerre M, et al. A global analysis of mucormycosis in France: the RetroZygo study (2005–2007). Clin Infect Dis 2012;54(S1):S35–43.
[3] Skiada A, Pagano L, Groll A, Zimmerli S, Dupont B, Lagrou K, et al. Zygomycosis in Europe: analysis of 230 cases accrued by the registry of the European Confederation of Medical Mycology (ECMM) Working Group on Zygomycosis between 2005 and 2007. Clin Microbiol Infect 2011;17:1859–67.
[4] Bitar D, Van Cauteren D, Lanternier F, Che D, Dromer F, Desenclos JC, et al. Increasing incidence of zygomycosis (mucormycosis), France, 1997–2006. Emerg Infect Dis 2009;15:1395–401.
[5] Kontoyiannis DP, Azie N, Franks B, Horn DL. Prospective antifungal therapy (PATH) alliance: focus on mucormycosis. Mycoses 2014;57:240–6.
[6] Ruping MJGT, Heinz WJ, Kindo AJ, Rickerts V, Lass-Florl C, Beisel C, et al. Forty-one recent cases of invasive zygomycosis from a global clinical registry. J Antimicrob Chemother 2010;65:296–302.
[7] Chakrabarti A, Singh R. Mucormycosis in India: unique features. Mycoses 2014;57(S3):85–90.
[8] Lelievre L, Garcia-Hermoso D, Abdoul H, Hivelin M, Chouaki T, Toubas D, et al. Posttraumatic mucormycosis. A nationwide study in France and review of the literature. Med (Balt) 2014;93:395–404.
[9] Slavin MA, Chakrabarti A. Opportunistic fungal infections in the Asia-Pacific region. Med Mycol 2012;50:18–25.
[10] Austin CL, Finley PJ, Mikkelson DR, Tibbs B. Mucormycosis: a rare fungal infection in tornado victims. J Burn Care Res 2014;35:e164–71.
[11] Cheng VCC, Chan JFW, Ngan AHY, To KKW, Leung SY, Tsoi HW, et al. Outbreak of intestinal infection due to *Rhizopus microsporus*. J Clin Microbiol 2009;47:2834–43.
[12] Chakrabarti A, Das A, Mandal J, Shivaprakash MR, George VK, Tarai B, et al. The rising trend of invasive mucormycosis in patients with uncontrolled diabetes mellitus. Med Mycol 2006;44:335–42.
[13] Slavin M, van Hal S, Sorrell TC, Lee A, Marriott DJ, Daveson K, et al. Invasive infections due to filamentous fungi other than *Aspergillus*: epidemiology and determinants of mortality. Clin Microbiol Infect 2015;21. 490.e1–10.
[14] National Centre for Classification in Health. International statistical classification of disease and related health problems. 10th revision. Australian modification (ICD-10-AM). Sydney: The Centre, University of Sydney; 1998.
[15] De Pauw B, Walsh TJ, Donnelly JP, Stevens DA, Edwards JE, Calandra T, et al. European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group; National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. Revised definitions of invasive fungal disease from 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 (EORTC/MSG) Consensus Group. Clin Infect Dis 2008;46:1813–21.
[16] Ciardo DE, Lucke K, Imhof A, Bloemberg GV, Bottger EC. Systematic internal transcribed spacer sequence analysis for identification of clinical mould isolates in diagnostic mycology: a 5-year study. J Clin Microbiol 2010;48:2809–13.
[17] Kurtzman CP, Robnett CJ. Identification of clinically important ascomycetous yeasts based on nucleotide divergence in the 5' end of the large-subunit (26S) ribosomal DNA gene. J Clin Microbiol 1997;35:1216–23.
[18] Lau A, Chen S, Sorrell T, Carter D, Malik R, Martin P, et al. Development and clinical application of a panfungal PCR assay to detect and identify fungal DNA in tissue specimens. J Clin Microbiol 2007;45:380–5.
[19] Park BJ, Pappas PG, Wannemuehler KA, Alexander BD, Anaissie EJ, Andes DR, et al. Invasive non-aspergillus mold infections in transplant recipients, United States, 2001–2006. Emerg Infect Dis 2011;17:1855–64.
[20] Ambrosioni J, Bouchuiguir-Wafa K, Garbino J. Emerging invasive zygomycosis in a tertiary care centre: epidemiology and associated risk factors. Int J Infect Dis 2010. 14S:e100–3.
[21] Gomes MZR, Lewis RE, Kontoyiannis DP. Mucormycosis caused by unusual mucormycetes, non-*Rhizopus*, -*Mucor*, and -*Lichtheimia* species. Clin Microbiol Rev 2011;24:411–45.
[22] Royer M, Puechal X. Mucormycosis in systemic autoimmune disease. Joint Bone Spine 2014;81:303–7.
[23] Ingram PR, Suthananthan AE, Rajan R, Pryce TM, Sieunarine K, Gardam DJ, et al. Cutaneous mucormycosis and motor vehicle accidents: findings from an Australian case series. Med Mycol 2014;52:819–25.
[24] Cornely OA, Arikan-Akdagli S, Dannaoui E, Groll AH, Lagrou K, Chakrabarti A, et al. ESCMID and ECMM joint clinical guidelines for the diagnosis and management of mucormycosis 2013. Clin Microbiol Infect 2014;S3:5–26.
[25] Lackner M, Caramalho R, Lass-Florl C. Laboratory diagnosis of mucormycosis: current status and future perspectives. Future Microbiol 2014;9:683–95.
[26] Abotlins CA, Pratt WAB, Solano TR. Fungemia secondary to gastrointestinal *Mucor indicus* infection. Clin Infect Dis 2006;42:154–5.
[27] Chan-Tuck KM, Nemoy LL, Perencevich EN. Central venous catheter-associated fungemia secondary to mucormycosis. Scand J Infect Dis 2005;37:925–7.