Invasive infections due to filamentous fungi other than *Aspergillus*: epidemiology and determinants of mortality

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Abstract

The epidemiology of invasive fungal disease (IFD) due to filamentous fungi other than *Aspergillus* may be changing. We analysed clinical, microbiological and outcome data in Australian patients to determine the predisposing factors and identify determinants of mortality. Proven and probable non-*Aspergillus* mould infections (defined according to modified European Organization for Research and Treatment of Cancer/Mycoses Study Group criteria) from 2004 to 2012 were evaluated in a multicentre study. Variables associated with infection and mortality were determined. Of 162 episodes of non-*Aspergillus* IFD, 145 (89.5%) were proven infections and 17 (10.5%) were probable infections. The pathogens included 29 fungal species/species complexes; mucormycetes (45.7%) and *Scedosporium* species (33.3%) were most common. The commonest comorbidities were haematological malignancies (HMs) (46.3%) diabetes mellitus (23.5%), and chronic pulmonary disease (16%); antecedent trauma was present in 21% of cases. Twenty-five (15.4%) patients had no immunocompromised status or comorbidity, and were more likely to have acquired infection following major trauma ($p < 0.01$); 61 (37.7%) of cases affected patients without HMs or transplantation. Antifungal therapy was administered to 93.2% of patients (median 68 days, interquartile range 19–275), and adjunctive surgery was performed in 58.6%. The all-cause 90-day mortality was 44.4%; HMs and intensive-care admission were the strongest predictors of death (both $p < 0.001$). Survival varied by fungal group, with the risk of death being significantly lower in patients with dematiaceous mould infections than in patients with other non-*Aspergillus* mould infections. Non-*Aspergillus* IFD affected diverse patient groups, including non-immunocompromised hosts and those outside traditional risk groups; therefore, definitions of IFD in these patients are required. Given the high mortality, increased recognition of infections and accurate identification of the causative agent are required.

Keywords: Determinants of outcome, epidemiology, filamentous fungus, non-*Aspergillus* moulds, predisposing factors
Original Submission: 14 November 2014; **Revised Submission:** 18 December 2014; **Accepted:** 30 December 2014
Editor: E. Roilides
Article published online: 14 January 2015

Clin Microbiol Infect 2015; **21:** 490.e1–490.e10

http://dx.doi.org/10.1016/j.cmi.2014.12.021

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Introduction

The frequency of invasive fungal disease (IFD) due to filamentous fungi, or moulds, other than *Aspergillus* is increasing. Although most infections have occurred in immunosuppressed patients, immunocompetent hosts may be affected [1–5]. Recent reports of infections following natural disasters [6] and case clusters following iatrogenic inoculation [7,8] highlight the potential of these environmental pathogens to cause serious infection in healthy hosts. The epidemiology of IFD is changing. The frequency of mucormycosis complicating diabetes mellitus has increased, with the emergence of species such as *Apophysomyces elegans* [9]. *Scedosporium*, including more recently designated species [5], phaeohyphomycetes such as *Verruconis gallopava* (previously *Ochroconis gallopava*) and the genus *Rasamsonia* [10–14] have been reported in distinct regions or patient populations. The intrinsic resistance of many non-*Aspergillus* moulds to marketed antifungal agents is of concern [15].

Many case series or reviews of non-*Aspergillus* mould infections have focused on specific patient populations, e.g. solid organ transplant recipients, or particular infections, such as phaeohyphomycosis, and have been limited by the lack, or limited use, of molecular identification techniques [1,3,4,10]. We performed this multicentre study of non-*Aspergillus* mould infections in Australian tertiary hospitals to better understand the current epidemiology, predisposing factors for and outcomes of these infections.

Methods

Study design

A retrospective multicentre, observational study of non-*Aspergillus* mould infections in adults from 2004 to 2012 was conducted through the Australia and New Zealand Mycoses Interest Group (ANZMIG). Human Research Ethics Committee approval was obtained at all study sites.

Data collection

Cases were identified through interrogation of microbiology and pathology laboratory information systems, infectious diseases databases, and hospital medical records, according to the International Statistical Classification of Diseases and Related Health Problems, 10th revision, Australian Modification (ICD-10-AM) for fungal disease and comorbidities [16]. Trained personnel collected data on standardized clinical record forms. Cases were reported to a central registry and reviewed by a data review committee (S.C., M.S., S.v.H., T.C.S., and A.L.) to determine the inclusion criteria for: (a) incident infection in unique (separate) patients, and (ii) only proven or probable IFD (see 'Definitions'). Cases classified by the data review committee as 'colonization' or as possible IFD were excluded.

Data on patient demographics and comorbidities (e.g. malignancy, transplantation, and diabetes mellitus), classic risk factors for IFDs (e.g. immunosuppressive therapies and intensive-care unit (ICU) stay) [2–5,9,17] and other possible predisposing factors (e.g. major trauma), clinical manifestations, laboratory and radiological results and antifungal and other therapies were collected. Patients were followed for a period of 6 months (180 days) from the time of IFD diagnosis or until death. Clinical outcomes, including dates of death, were recorded.

Definitions

Definitions of proven or probable non-*Aspergillus* IFD were adapted from the European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) definitions for IFD [18]. IFD was proven when fungal elements were visualized microscopically within tissue, or a mould was cultured from a sterile site, for a patient with clinical and radiological features of infection. A probable infection was defined by culture of a mould from a non-sterile site, including bronchoalveolar lavage fluid, in a patient with clinical and radiological evidence of IFD; host-specific criteria were not required (in contrast to the EORTC/MSG criteria for probable IFD [18]).

Infection was defined as disseminated when two or more non-contiguous sites were involved or blood cultures were positive. Major trauma referred to significant tissue injury requiring surgical intervention. The primary clinical outcome was assessed as 90-day all-cause mortality, in order to allow comparison of outcomes with those of other studies [2,19]. Secondary outcomes included all-cause mortality at 30 days and at the end of follow-up (180 days). Physician-ascribed attributable mortality was also assessed.

Microbiology

Cultures and histopathological specimens were examined at participating sites. Fungi were identified with standard phenotypic methods. Ninety isolates (63.4%) were also subjected to species confirmation by DNA sequencing, 80 at one of two reference mycology laboratories (Westmead Hospital, Sydney; SA Pathology, Adelaide), with targeting of the internal transcribed spacer regions or the D1/D2 regions of the 28S (large

subunit) of the rRNA gene [20,21]. In addition, the gene for the translation elongation factor EF1α was sequenced for identification of *Fusarium* species [22].

When a fungus was visualized in tissue and other sterile site specimens but not cultured, internal transcribed spacer sequencing [23] was performed for identification. When species assignment could not be made, or when sequencing was technically unsuccessful, isolates were categorized by genus (e.g. *Rhizopus*) or class (e.g. agent of mucormycosis).

Statistical analysis

Associations between fungal species and patient comorbidity or predisposing factors for non-*Aspergillus* IFDs were analysed. Determinants of all-cause 90-day mortality for all infections, and for subgroups of non-*Aspergillus* IFD (grouped by mucormycete, *Scedosporium*, *Fusarium* and dematiaceous mould infections) were also examined. Categorical variables were compared by use of the chi-square test or Fisher's exact test, and continuous variables by the use of Student's *t*-test or the Mann–Whitney *U*-test. Multivariate backward stepwise logistic regression analysis was performed to identify independent predictors of disseminated infection and of mortality for the entire study cohort and for subgroups of infection (as above). Variables with a p-value of <0.20 by univariate analysis were included in the model. Kaplan–Meier survival analysis was performed for all-cause 90-day mortality. Calculations were performed with SPSS version 22.0 (SPSS, Chicago, IL, USA).

Results

One hundred and eighty non-*Aspergillus* mould infections were reported from 15 tertiary hospitals. All jurisdictions other than Queensland and Tasmania were represented. There were no case clusters. Following review, 162 of 180 episodes of infection (162 patients) met the inclusion criterion of proven or probable IFD. There were 145 (89.5%) proven and 17 (10.5%) probable infections. There was no trend in the annual frequency of cases (range 12–24).

Causative fungi

Twenty episodes of infection were diagnosed by histopathology alone. Of 142 culture-positive episodes, diagnosis was made by culture alone in 76 (53.5%) and with tissue histopathology in 66 (46.5%). DNA sequencing was performed in 91 of 142 (64.1%) culture-positive episodes and in ten (50%) histologically confirmed cases. In referred samples (n = 80), the final identification was considered to be that obtained by the reference laboratory, including those for which species identification was discordant (3.8% of cases; data not shown).

Overall, 112 isolates (69.1%) were identified to species level and 38 (23.5%) to genus level. The remaining 12 cases were all attributed to mucormycetes following visualization of characteristic hyphae on histopathology.

Infections were caused by a range of pathogens. Mucormycetes accounted for 74 of 162 (45.7%) episodes, followed by *Scedosporium* (33.3%); *Fusarium* species caused 8% of cases, and there were four infections caused by very uncommon moulds (Table 1). Three patients had mixed infections, one each due to *Fusarium* and *Paecilomyces*, *Fusarium* and *Mucor*, and *Exserohilum* and *Scedosporium* species.

Most mucormycetes (54%; Table 1) were from the genus *Rhizopus*. *Scedosporium prolificans* accounted for ~50% of *Scedosporium* infections. Of ten phenotypic '*Scedosporium apiospermum*' isolates, two were *Scedosporium aurantiacum* by DNA sequencing; however, as not all *Scedosporium* isolates were subjected to molecular identification, isolates will hereafter be referred to as '*S. prolificans*' or '*S. apiospermum*/*Pseudallescheria boydii* species complex'. Dematiaceous fungi were diverse (Table 1). The pattern of pathogens was similar across institutions, and was independent of location, with the exception of environmental fungi (*Ganoderma* species and *Lasiodiplodia*

TABLE 1. Causative pathogens: non-*Aspergillus* mould infections, Australia 2004–2012

Organism	No. of isolates	No. identified by DNA sequencing
Mucormycetes	74	—
Apophysomyces species	4	3
Lichtheimia species	2	2
Mucor species	7	4
Rhizopus species	40	24
Rhizomucor species	4	4
Saksenaea species	4	3
Unclassified mucormycete	12[a]	—
Scedosporium species	54	—
Scedosporium apiospermum/*Pseudallescheria boydii* species complex	25[b]	10
S. prolificans	29	5
Fusarium species	13	—
F. solani complex	7	4
F. oxysporum complex	1	1
Unclassified	5[c]	5
Other hyphomycete fungi	4	—
Phialemonium species	1	1
Paecilomyces species	3	3
Dematiaceous fungi	16	—
Acrophialophora species	1	—
Alternaria species	1	1
Bipolaris species	2	1
Chaetomium species	1	1
Cladophialophora species	1	1
Curvularia species	2	1
Exserohilum species	2	1
Exophiala species	1	—
Fonsacaea species	1	—
Microsphaeropsis species	1	1
Verruconis species	2	2
Phialophora species	1	1
Other	4[d]	2

[a]See text for details.
[b]Includes two cases of *S. aurantiacum* infection: one ocular infection resulting in enucleation of the eye, and one case of cerebral abscess.
[c]Identified as *Fusarium* species by DNA sequencing.
[d]*Ganoderma* species, *Lasiodiplodia* species, *Trichoderma longibrachiatum*, and *Thermomyces lanuginosus*.

species) that caused post-inoculation injury infections in tropical northern Australia.

Patient characteristics and comorbidities

The median age of patients was 57 years (interquartile range (IQR) 45–65 years); 108 (66.7%) were male. Although infection peaked in patients aged 60–70 years, the distribution of aetiological fungi was similar across age groups (Fig. 1). The number of cases varied by jurisdiction, reflecting the population density and medical services at each institution (data not shown).

Patient comorbidities and predisposing factors for non-*Aspergillus* mould infections are shown in Table 2. Of all infections, 37.7% occurred in patients without haematological malignancy (HM) cancers, haematopoietic stem cell transplantation (HSCT), or solid organ transplantation (SOT). No patient with diabetes mellitus (n = 38; 23.5%) had concurrent ketoacidosis. Thirty-four patients (21%) had antecedent traumatic injuries. Over 50% of patients receiving antifungal prophylaxis (39.5% of all patients) were taking voriconazole or posaconazole. Twenty-five patients (15.4%) had neither underlying immunocompromised status nor comorbidity; they were more likely than the remaining 137 to have acquired infection through major trauma (8/25 (32%) vs. 8/137 (5.8%), p <0.01).

TABLE 2. Patient comorbidities and predisposing factors in 162 episodes of non-*Aspergillus* mould infection

Characteristic	No. of episodes (%)
No comorbidity or host immunocompromised status	25 (15.4)
Underlying comorbid conditions	
Diabetes mellitus	38 (23.5)
Chronic obstructive pulmonary disease	26 (16)
Chronic renal failure	17 (10.5)
Rheumatological conditions	14 (8.6)
Haematological malignancy[a]	75 (46.3)
Stem cell or bone marrow transplant	27 (16.7)
Solid organ transplant	23 (14.2)
Solid malignancy	7 (4.3)
Predisposing factors	
Major or minor trauma within the preceding 30 days	34 (21)
Major surgery within the preceding 30 days	32 (19.8)
Hospital construction within the preceding 30 days	30 (18.5)
Cancer chemotherapy	69 (42.6)
Corticosteroids	88 (54.3)
Monoclonal antibodies	18 (11.1)
Calcineurin inhibitors	34 (21)
Neutropenia within the preceding 30 days	64 (39.5)
Other patient characteristics	
Antifungal prophylaxis	64 (39.5)
Fluconazole	17 (11.1)
Itraconazole	7 (4.3)
Voriconazole	13 (8.0)
Posaconazole	20 (12.3)
Liposomal amphotericin B	3 (1.9)
Current smoker	37 (22.8)
Intensive-care unit admission at time of diagnosis of fungal infection	59 (36.4)

[a]Haematological cancers included: acute leukaemia (n = 42), chronic leukaemia (n = 8), lymphoma (n = 8), multiple myeloma (n = 4), myelodysplastic syndrome (n = 8), and other cancers (n = 5).

Comorbidity, predisposing factors, and causative pathogens

The influence of clinical variables on IFD aetiology is shown in Table 3. Diabetes mellitus as such did not select for a mucoraceous infection. As compared with infections due to non-mucormycetes (n = 88), there was an association between mucormycosis and voriconazole prophylaxis (13.5% (10/74) vs. 3.4% (3/88), p 0.022). As compared with non-*Fusarium* infections (n = 149), cases of fusariosis (n = 13) were significantly more common in neutropenic patients (18.8% vs. 1%, p <0.001) and HM patients (16% vs. 1.1%, p 0.001). Scedosporiosis (n = 54) was more common post-SOT (52.2% (12/23) vs. 30.2% (42/39), p 0.039) than in non-organ transplant recipients.

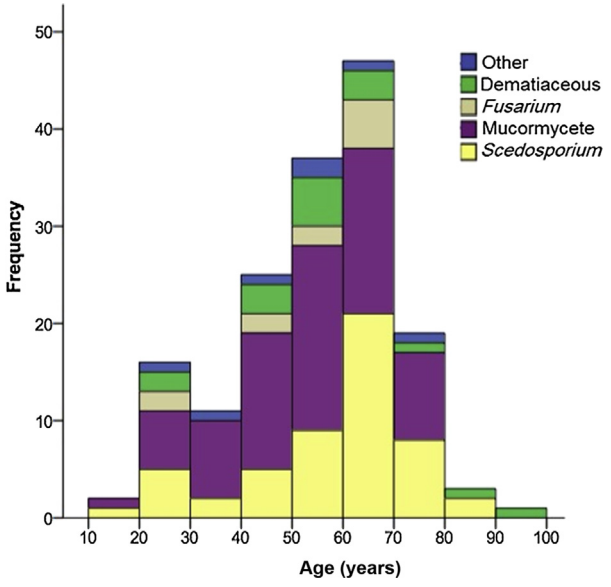

FIG. 1. Distribution of non-*Aspergillus* mould infection episodes according to age and fungal genera.

Site of infection

Sixty-one patients (37.7%) presented with disseminated infection, and 101 (62.3%) with localized infection. Diverse anatomical sites were affected (Fig. 2), but, overall, the lung was the most common (40.1%). Of 17 blood isolates, 14 were *S. prolificans*, two were *Fusarium solani*, and one was a mucormycete. Four patients with HM/HSCT were diagnosed with isolated fungaemia (all *S. prolificans*). Eleven of 19 cases of brain infection were caused by a mucormycete, and six by *Scedosporium* species. All 15 eye infections manifested as endophthalmitis (50% due to *Scedosporium*). The two known *S. aurantiacum* infections presented as localized eye and brain disease. Four cases of endocarditis complicated by culture-positive endovascular infection were due to *Thermomyces lanuginosus* (n = 1), mucormycetes (n = 2), and *Trichoderma longibrachiatum* (n = 1).

The gastrointestinal tract, heart, liver and deep arterial thrombi (Fig. 2) were involved only in disseminated infection,

TABLE 3. Non-*Aspergillus* mould pathogen (*n* = 165 isolates) groups according to major patient co-morbidity and predisposing factors

Variable	Mucormycete (*n* = 74)	*Scedosporium* (*n* = 54)	*Fusarium* (*n* = 13)	Dematiaceous molds (*n* = 16)	Other (*n* = 8)
Co-morbidity					
None (*n* = 25)[a]	8	9	1	4	3[b]
Diabetes (*n* = 38)	20	13	1	4	1
Chronic lung disease (*n* = 26)	11	11	0	3	1
Hematologic malignancy (*n* = 75)	36	23	12[c]	5	1
Solid organ cancer (*n* = 7)	2	4	0	1	0
Stem cell transplant (*n* = 27)	13	9	3	3	0
Solid organ transplant (*n* = 23)	8	12[c]	0	3	1
Predisposing factor					
Neutropenia (*n* = 64)	33	18	11[c]	3	1
Major or minor trauma (*n* = 33)	17	10	2	4	3[d]
Building construction (*n* = 30)	10	12	3	2	3
Medications prescribed[e]					
Antifungal prophylaxis					
Any Agent (*n* = 64)	34	19	10	3	0
Voriconazole (*n* = 13)	10[c]	3	0	0	0
Posaconazole (*n* = 20)	10	7	4	0	0
Corticosteroids (*n* = 88)	40	29	10	8	4
Monoclonal antibody (*n* = 18)	7	7	3	1	1

[a]Also no host immunocompromise.
[b]*Paecilomyces*, *Trichoderma* and *Thermomyces* species.
[c]See text for association details.
[d]*Lasiodiplodia*, *Phialemonium*, and *Paecilomyces* species.
[e]Medications prescribed within the preceding 30 days.

with the eye being the only other site associated with disseminated disease (16.4% (10/61) of cases vs. 5.1% (5/101) of cases not affecting the eye, p 0.02). Underlying HM independently predicted disseminated infection (OR 2.7, 95% CI 1.4–5.3, p 0.03; Table 4). Dematiaceous moulds were associated with localized infections.

Patient management

Antifungal treatment was initiated in 151 patients (93.2%) and continued for a median of 68 days (IQR 19–275 days); 114 (74.5%) patients received monotherapy. Dual-agent and three-agent antifungal therapy were used in 34 and three patients, respectively. Antifungal therapy was largely determined by the fungal pathogen. Amphotericin B monotherapy (predominantly liposomal amphotericin B) was typically used to treat mucormycoses (67.6%; 60/74 cases), and voriconazole alone (*n* = 29) or in combination with terbinafine (*n* = 13) was predominantly prescribed for *Scedosporium* infections (77.8%; 42/54 cases).

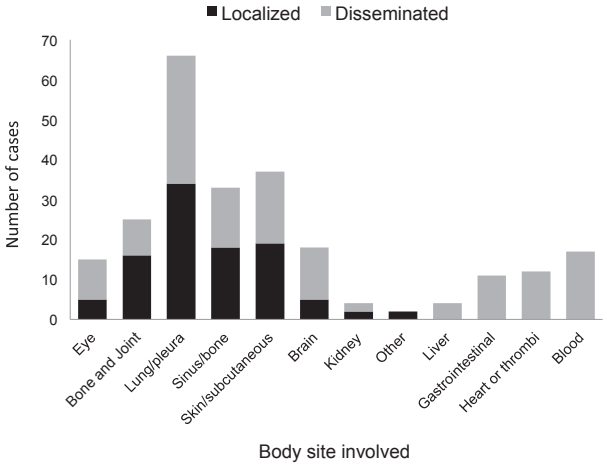

FIG. 2. Body site of infection in non-*Aspergillus* mould infections. Note that all involved sites are depicted in patients with disseminated disease (*n* = 61). Involvement of the liver, gastrointestinal tract, heart and deep arterial thrombi was exclusively associated with disseminated infections, as were bloodstream infections (by definition).

TABLE 4. Comparison of features of disseminated and localized non-*Aspergillus* mould infections in 162 patients

	Disseminated infection (*n* = 61), no. (%)	Localized infection (*n* = 101), no. (%)	p
Comorbidities			
None	8 (13.1)	17 (16.8)	0.66
Diabetes mellitus	15 (24.6)	23 (22.8)	0.79
Chronic lung disease	9 (14.8)	17 (16.8)	0.73
Haematological malignancy	38 (62.3)	37 (36.6)	0.002
Solid organ cancer	2 (3.3)	5 (5.0)	0.71
Stem cell transplant	14 (23.0)	13 (12.9)	0.095
Solid organ transplant	8 (13.1)	15 (14.9)	0.76
Predisposing factors			
Neutropenia	34 (55.7)	30 (29.7)	0.001
Major or minor trauma	11 (18.0)	23 (22.8)	0.47
Building construction	10 (16.4)	10 (19.8)	0.68
Medications received			
Cancer chemotherapy	35 (57.4)	34 (33.7)	0.003
Steroids	37 (60.7)	51 (50.5)	0.21
Monoclonal antibodies	10 (16.4)	8 (7.9)	0.10
Calcineurin inhibitors	13 (21.3)	21 (20.8)	0.94
Fungal pathogen[a]			
Mucormycete	29 (47.5)	45 (44.6)	0.71
Scedosporium	20 (32.8)	34 (33.7)	0.91
Fusarium	9 (14.8)	4 (4.0)	0.01
Dematiaceous mould	2 (3.3)	14 (13.9)	0.03
Other	2 (3.3)	6 (5.9)	0.71

[a]One patient had disseminated infection due to a mucormycete and *Fusarium*.

Adjunctive surgery was performed in 95 of 162 (58.6%) patients, and appeared to be determined by the site of infection rather than the pathogen. Adjunctive granulocyte–macrophage colony-stimulating factor or granulocyte infusion therapies were used in only four haematology patients.

Outcomes

The all-cause 90-day mortality was 44.4% (72/162 patients), with most (94.4%) deaths being attributable to IFD. Deaths occurred at a median of 14 days (IQR 5–28 days) after IFD diagnosis. All-cause 30-day and 180-day mortality were 35.8% (n = 58) and 47% (n = 77), respectively. Table 5 shows the variables associated with mortality by univariate and multivariate analyses. Underlying HM and ICU admission (both p <0.001) were the strongest independent predictors of outcome (see also Figs. 3(a) and (b)), but rheumatological disease and disseminated infection (both p 0.02) also increased the risk of death. The type of haematological cancer and neutropenia did not predict death. Surgery was associated with a survival advantage on multivariate analysis (OR 0.34, 95% CI 0.13–0.88, p 0.027).

Fungal type and outcome

Dematiaceous fungal infections were independently associated with reduced mortality (OR 0.1, 95% CI 0.02–0.9, p 0.04) (Fig. 3(c)) in the multivariate analysis of the entire dataset. When subgroup analysis examining mortality was performed for the fungal groups with sufficient numbers, namely the mucormycetes, *Scedosporium* species, and dematiaceous moulds, no fungal-specific risk factors for outcome that were different from the entire dataset were identified on either univariate or multivariate analysis. Specifically for mucormycte infections, being in an ICU at diagnosis (OR 6.6, 95% CI 1.4–29), both a diagnosis of acute leukemia (OR 17.8, 95% CI 3–103) and a diagnosis of of haematological cancer other than acute leukaemia (OR 29.5, 95% CI 4.5–196) and disseminated infection (OR 4.9, 95% CI 1.2–20.2) were all independent predictors of mortality.

Discussion

This multicentre study of non-*Aspergillus* filamentous fungal infections describes the epidemiology of these emerging pathogens in the era of molecular diagnostics and contemporary management protocols [24]. A broad range of fungi caused IFD. Although many patients had traditional predisposing factors, predominantly immunosuppression, >15% had no comorbidities and were not immunocompromised, and 37.7% were not undergoing treatment for HM or transplantation.

TABLE 5. Univariate and multivariate analysis of overall 90-day outcome in patients with non-*Aspergillus* mould infections

	Univariate analysis			Multivariate analysis		
Variable	**Died (n = 72)**	**Alive (n = 90)**	**p**	**OR**	**95% CI**	**p**
Age (years), median (IQR)	58 (40–76)	56 (33–79)	0.75	—	—	—
Male gender, no. (%)	48 (66.7)	60 (66.7)	1.00	—	—	—
Current smoker, no. (%)	12 (16.7)	25 (27.8)	0.14	—	—	—
Comorbidities/predisposing conditions, no. (%)						
Diabetes	12 (16.7)	26 (28.9)	0.07	—[a]	—	—
COPD	12 (16.7)	14 (15.6)	0.85	—	—	—
Chronic renal failure	2 (2.8)	15 (16.7)	0.004	—[a]	—	—
Rheumatological malignancy	10 (13.9)	4 (4.4)	0.05	7.1	1.30–38.1	0.02
Haematological malignancy	58 (77.3)	17 (18.9)	<0.001	33.5	10.52–10.48	<0.001
Bone marrow transplant	22 (30.6)	5 (5.6)	<0.001	—[a]	—	—
Solid organ transplant	5 (6.9)	18 (20.0)	0.02	—[a]	—	—
Solid malignancy	3 (4.2)	4 (4.4)	1.00	—	—	—
No comorbidities	1 (1.4)	24 (26.7)	<0.001	—[a]	—	—
Additional predisposing factors within the preceding 30 days, no. (%)						
Major trauma	4 (5.6)	12 (13.3)	0.1	—[a]	—	—
Surgery	15 (20.8)	17 (18.9)	0.76	—	—	—
Hospital construction	13 (18.1)	17 (18.9)	0.89	—	—	—
Medications prescribed						
Chemotherapy	47 (65.3)	22 (24.4)	<0.001	—[a]	—	—
Steroids	52 (72.2)	36 (40)	<0.001	—[a]	—	—
Monoclonal antibodies	15 (20.8)	3 (3.3)	<0.001	—[a]	—	—
Calcineurin inhibitors	13 (18.1)	21 (23.3)	0.41	—	—	—
Neutropenic	49 (68.1)	15 (16.7)	<0.001	—[a]	—	—
Manifestations of infection						
Localized infection	30 (41.7)	70 (77.8)	—	—	—	—
Disseminated infection	42 (58.3)	20 (22.2)	<0.001	3.2	1.2–8.4	0.02
Within ICU at the time of IFI	38 (52.8)	21 (23.3)	<0.001	7.5	2.4–23.2	<0.001
Fungal aetiology, no. (%)						
Mucormycetes	38 (52.8)	36 (40.0)	0.11	—[a]	—	—
Scedosporium species	25 (34.7)	28 (31.1)	0.63	—	—	—
Fusarium species	8 (11.1)	5 (5.6)	0.19	—	—	—
Dematiaceous mould	2 (2.8)	14 (15.6)	0.007	0.1	0.02–0.9	0.04

COPD, chronic obstructive pulmonary disease; IQR, interquartile range.
[a]Variables that were included in the multivariate analysis but dropped out of the final model.

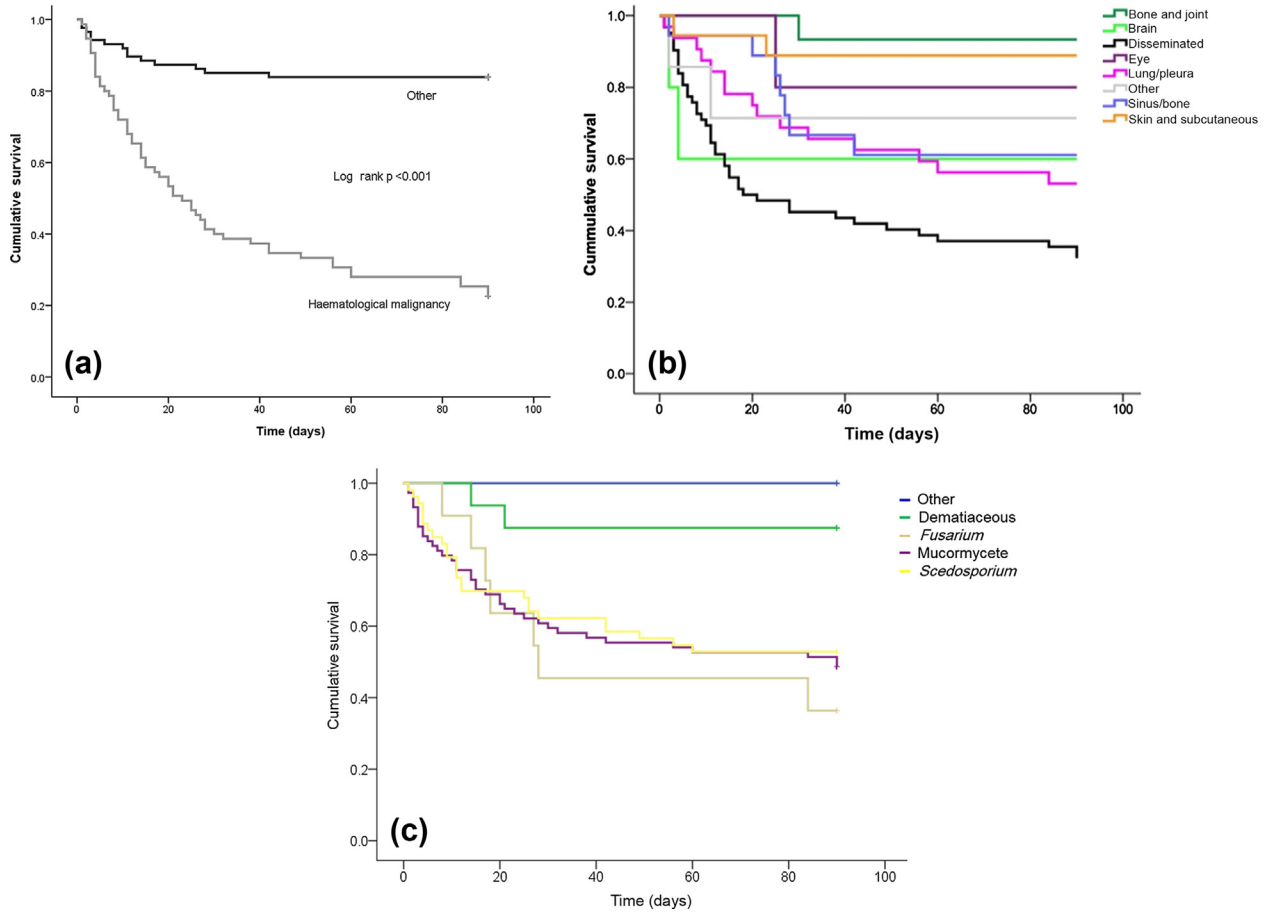

FIG. 3. (a) Kaplan–Meier survival curve stratified by the presence or absence of an active haematological malignancy. (b) Kaplan–Meier survival curve stratified by body site of infection: disseminated invasive fungal disease and different body sites of infection. (c) Kaplan–Meier survival curve stratified by classification of fungal genus.

Inclusion of multiple centres in this study enabled the recognition of 29 aetiological species/species complexes; because of the rarity of some species, results from single institutions or specific populations are unlikely to be representative. Mucormycetes were the leading cause of IFD (45.7%). As expected, we observed mucormycosis in HSCT, SOT and diabetes patients [2,17,25,26]. Notably, mucoraceous fungi were also the commonest causes of infection following various antecedent traumas, supporting a recent observation linking these infections to road trauma [27].

The epidemiology of non-*Aspergillus* moulds in this study differed from that in other global reports. Outside of Australia, *Fusarium* species are typically the second commonest non-*Aspergillus* pathogens after the mucormycetes, generally affecting haematology patients or SOT recipients [2,25,26,28]. Although fusariosis did occur in patients with HMs in our study, these infections were uncommon (8%). In contrast, the prominence of *Scedosporium* species (33.3%) reflects a relatively high incidence of these infections in Australia [5,28,29] as compared with other settings [2]. Chronic lung disease, SOT and trauma have been observed to be predisposing factors for scedosporiosis [3,5,30,31]. The reasons for these subtle differences in epidemiology are unknown, but may reflect the abundance of *Scedosporium* in the soil, especially in Australian urban environments [32]. It is possible that engaging in major horticulture/landscaping activities poses an added risk for scedosporiosis.

Another key finding is the range of patient populations affected outside those traditionally at high risk for IFD (i.e. HM and SOT recipients). Emerging at-risk populations included those with chronic lung disease, those with rheumatological conditions (presumably due to the increasing use of immune-modulating drugs such as tumour necrosis factor-α antagonists [33]), and those sustaining trauma. Indeed, inoculation injuries caused 34 (21%) of all infections. Although mucormycetes were common (51%), *Scedosporium* species and dematiaceous and other environmental moulds were also isolated, highlighting the pathogenic potential of these fungi after even

minor trauma. Notably, 15% of cases in our study were identified in patients without comorbidities or immunocompromised status. In a previous study of mucormycosis, 19% of patients had no apparent comorbidity [17]. It is of interest that mucormycosis represented only half of the infections in diabetic patients, suggesting that the epidemiology of non-*Aspergillus* mould infections is changing in this patient group [19]. Although voriconazole prophylaxis was associated with mucormycosis [2,34], it may represent a surrogate for an alternative mucormycosis risk factor rather than causation as such. However, our study was not designed to resolve this important issue.

Dematiaceous fungal infections remain uncommon, but appear to be emerging in a diverse group of patients, including normal hosts after trauma. Two *V. gallapova* infections were observed, both in neutropenic haematology patients with adverse outcomes despite combination therapy with posaconazole or voriconazole and amphotericin B, all of which have, in general, good *in vitro* activity against *V. gallopava* [35]. Nonetheless, overall, dematiaceous moulds were associated with lower mortality than other non-*Aspergillus* IFDs, which is similar to recent findings of phaeomycotic infections in SOT recipients (7% mortality), although most of these had skin rather than disseminated infection [10]. In contrast, there was a 33% mortality rate in patients with proven/probable dematiaceous mould infections in a cancer centre, and dissemination was identified as a risk for mortality [1]. These outcome differences probably reflect variability in host immunosuppression and site of infection.

As almost 40% of our cases occurred outside the haematology and transplant patient population, there is a clear need to extend definitions of non-*Aspergillus* mould IFD beyond these patient cohorts. Although the majority of our cases (89.5%) were proven infections, our assignment of probable IFD cases outside of recognized high-risk groups was arbitrary, albeit based on modified EORTC/MSG definitions [18], in that patients with probable IFD did not have to meet prespecified host criteria. Assignment of 'possible' IFD, especially in this group, was not feasible. Two factors impact on the criteria for categorizing these IFDs. The first is the lack of accessible, rapid methods to detect non-*Aspergillus* moulds in bronchoalveolar lavage fluid, blood, and other specimens. The US outbreak of *Exserohilum rostratum* meningitis illustrates the importance of access to rapid tests, with diagnostics relying on the development of relevant PCR assays [36] in addition to application of the β-D-glucan test [37] for pathogen or biomarker detection in clinical specimens. Second, despite the ability to develop diagnostic tests, probable and possible IFD remain dependent on host criteria.

Almost 40% of patients had disseminated infection. In particular, gastrointestinal tract, liver or eye involvement should alert the clinician to look for other sites of infection. The burden of infection was also appreciable, with a 90-day mortality of 44%. This underscores the need for validated, rapid diagnostics to improve clinical outcomes [38]. Selection of early and appropriate antifungal therapy is essential, as non-*Aspergillus* moulds have variable antifungal susceptibilities [14,15,35]. Management of these infections was complex and prolonged. Extended antifungal therapy, combined with surgery, was required in 58.6% of patients, and 36% required ICU care. This is pertinent, as ICU admission independently predicted mortality, as did HM, rheumatological conditions, and disseminated infection. Conversely, adjunctive surgery conferred a survival advantage. Within fungal groups, we were unable to determine independent predictors of mortality, owing to insufficient patient numbers, except in patients with mucormycosis, where predictors of death were similar to those for the whole patient cohort. The overall all-cause mortality was similar to that found in other series of diverse patient populations [2,3,15,19].

The study was limited by its retrospective nature, possible ascertainment bias, and unexpectedly low numbers of probable IFDs. The last of these may reflect the difficulty in diagnosing probable (and probably true) infection in the absence of accepted diagnostic criteria, or a lack of sufficient diagnostic effort. The diagnostic algorithms employed at the 15 contributing sites may have differed. Not all isolates underwent DNA sequencing, so the occurrence of new/cryptic species was probably underestimated, especially among *Fusarium* species and the *S. apiospermum*/*P. boydii* species complex. The low numbers of rare pathogens limits the ability to perform subgroup analyses. As not all Australian jurisdictions provided data, we cannot estimate the national burden of these infections.

In conclusion, this study provides contemporary information about the spectrum of non-*Aspergillus* moulds causing IFDs and the affected patient groups. Mortality remains high despite aggressive antifungal therapy and surgery, although adjunctive therapy was associated with a survival advantage. Early diagnosis of these infections is needed to improve outcomes. Definitions of IFD in non-immunocompromised hosts should be developed.

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Transparency declaration

S. Chen, M. Slavin, S. van Hal, C. Heath, D. Marriott, N. Bak, C. Orla Morrissey and T. Sorrell are on the Antifungal Advisory Boards of Gilead Sciences Inc., MSD Australia, and Pfizer Australia. S. Kidd is on the Antifungal Advisory Board of Pfizer Australia and Mayne Pharma. T. Korman is on the Antibacterial Advisory Board of Pfizer Australia. T. Sorrell, D. Marriott, M. Slavin, S. Chen, S. van Hal, C. Heath, N. Bak, C. Orla Morrissey, S. Kidd and W. Meyer have received funding in the form of untied grants from Gilead Sciences Inc., MSD Australia, and Pfizer Australia. C. Halliday and S. Kidd have received funding in the form of untied educational grants from Merck. T. Korman has received monetary reimbursement for service on speakers' bureaus for Novartis (2010) and Merck (2011). S. Chen has received monetary reimbursement for service on speakers' bureaus from Gilead Sciences Inc. (2014). S. Kidd has received monetary reimbursement for service on speakers' bureaus from Gilead Sciences Inc. (2013). Monetary reimbursements from MSD Australia (2013), Pfizer Australia (2010) and Gilead Sciences Inc. (2012–1014) to C. Orla Morrissey for Speaker's Bureau were paid to Alfred Health

Acknowledgements

We thank hospital scientists across Australia for their assistance in fungal identification, and in the forwarding of isolates to the reference laboratories. We also thank A. Riddell, based at the Royal Darwin Hospital when the study was performed, and C. Mahoney and N. Cornish of Deakin University, for their assistance in data collection. T. C. Sorrell is a Sydney Medical School Foundation Fellow whose work is funded by the National Health and Medical Research Council of Australia (#1050106). The authors thank Pfizer Australia, Gilead Sciences Inc. and Merck for their financial support, in part, for this study.

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