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Title: Mucormycete Infection or Colonisation: Experience of an Australian Tertiary Referral Centre

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Abstract

Background: Mucormycosis is associated with significant morbidity and mortality. We reviewed patients with mucormycete isolated at Alfred Health, Australia.

Methods: A retrospective review of 66 patients with mucormycete(s) identified, between 1 April 2008 and 30 June 2014. Baseline demographic, microbiological, radiological, treatment/outcome data were recorded.

Results: Site of isolation was sino-pulmonary in 77% and skin/soft tissue in 21%. Thirty-two percent of cases were proven-IFD, 12% probable-IFD and 56% were defined as no-IFD (or colonization). *Rhizopus* spp. was identified in 48%. Comparing probable/proven-IFD with no-IFD/colonisation, more patients were post allogeneic stem cell transplantation (28% vs. 0%,p<0.01) and were receiving immunosuppressive therapy (59% vs. 24%,p<0.01) including prednisolone >20mg daily (24% vs. 5%,p=0.04).

Ninety-three percent of patients with proven/probable IFD received treatment whilst 30% of no-IFD/colonisation were treated. Seventy-two percent of patients with proven/probable IFD and 92% of those with colonisation had no further mucormycete isolated. Thirty-day mortality was higher in the proven/probable-IFD cohort (24%) compared with no-IFD/colonisation (3%) (p=0.02).

Conclusions

Mucormycosis remains uncommon, with 56% of cases not associated with clinical infection. Immunosuppressive therapy remains strongly associated with mucormycosis. Mortality remains high in those with proven/probable IFD.

Introduction

Mucormycosis (zygomycosis) is associated with significant morbidity and mortality, especially amongst immunocompromised hosts. Mucormycosis in trauma and burns patients is also increasingly recognized [1, 2]. Although initially considered a rare infection associated with close to 100% mortality, the incidence and prognosis of this infection is likely to have changed with the advent of newer triazole and lipid amphotericin based therapies [3, 4]. The objective of this study was to review the current clinical features, risk factors, treatment and outcomes in patients with mucormycosis diagnosed at Alfred Health, Melbourne, Australia in the setting of new antifungal agents.

Methods

Study design and setting

We analysed retrospectively collected data from patients who had mucormycetes identified by culture, PCR or histopathological methods at Alfred Health between 1 April 2008 and 30 June 2014. Alfred Health is a tertiary referral center, encompassing statewide (Victoria) Trauma, Burns, HIV, Cystic fibrosis (CF), Heart/Lung and Bone Marrow Transplant services. Mucormycosis was defined as an infection caused by (i) *Rhizopus* spp., (ii) *Rhizomucor* spp., (iii) *Mucor* spp., (iv) *Saksenaea* spp., or (v) mucormycete/mucormycosis, not otherwise speciated (i.e. histopathological detection only/PCR not technically possible). Fungi were identified by standard phenotypic methods and panfungal PCR as previously described. [5] Ethics approval was obtained from the Alfred Health Human Research Ethics Committee.

Study population

Data from patients with a mucormycetes isolated from culture, detected by pan-fungal PCR or reported from histopathology specimens were recorded. The specimen type, site and date of isolation were documented. Patient baseline characteristics, residence, underlying co-morbidities, immunosuppression and anti-fungal therapy details were recorded. Rural addresses were classified as any postcode outside of the Melbourne greater metropolitan area (as defined by Australia Post). Inpatient mortality was recorded.

Definitions

Definitions of proven and probable mucormycete infection were adapted as per Slavin et al. from the European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) definitions for IFD [6, 7]. Cases that did not fulfil the modified EORTC/MSG criteria were deemed to have colonisation (no-IFD). In lung transplant recipients, colonisation was defined as per Infectious Diseases Working Group of the International Society for Heart and Lung Transplantation (ISHLT) [8]. No recurrence (clinical cure) was defined as no further detection of mucormycetes by histopathology or microbiology following cessation of treatment dose antifungal therapy at last follow-up (i.e. 60-days post IFD diagnosis or at death, if earlier). In cases of colonization/no-IFD, no recurrence was defined as the absence of repeat positive microbiology or histopathology results. As a matter of routine practice chronic respiratory patients undergo routine surveillance sputum or bronchoscopy and trauma/burns patients surveillance tissue cultures. Patients with chronic respiratory disease included those with cystic fibrosis, interstitial pulmonary fibrosis, chronic obstructive pulmonary disease and lung transplant recipients.

Statistical analysis

Categorical variables were summarized using frequency and percentage and compared between groups using a Fischer's exact test. Continuous variables were summarized using mean and standard deviation (SD) or median and inter-quartile range (IQR), as appropriate and compared using a paired t-test or Wilcoxon signed-rank test, as appropriate. A p-value < 0.05 was considered statistically significant.

Results

We identified 66 patients where mucormycetes were detected. One patient had two distinct infections with different species (*Rhizopus spp* and *Rhizomucor spp*) isolated greater than two years apart. This was categorized as two separate infections. Baseline characteristics for the cohort and differences in baseline demographics between proven/probable-IFD and colonisation cases are outlined in Table 1.

Seventy three percent of patients were male (48/66) and the median age was 56.5 years. In fifteen cases (23%) mucormycetes were isolated in patients that were never investigated or treated in the inpatient setting. Sino-pulmonary (77%) was the most

common site of isolation (Table 1). All patients had a least one clinical specimen provided post initial mucorrmycete identification.

Mucormycetes were detected by culture alone in 71% (47/66), histopathology alone in 8% (5/66) and by the combination of panfungal PCR and histopathology in 9% (6/66). Overall, 70% (46/66) of mucormycetes were identified to species level and 21% (14/66) to genus level and 9% (6/66) to class level (i.e. mucormycete) following visualisation of characteristic hyphae on histopathology. Of the cases identified to class level only, 3 (33%) were further speciated using panfungal PCR. *Rhizopus* spp. was isolated in 48% (32/66), followed by *Rhizomucor* in 29% (19/66) and *Mucor* spp. in 12% (8/66). *Saksenaea vasiformis* was isolated in one patient. There were 21 proven-IFD, 8 probable-IFD and 37 cases with colonisation (no IFD). The primary site of IFD and colonisation was respiratory, 55% (16/29) and 84% (31/37) respectively. In proven/probable-IFD diagnosis was made via culture alone in 41% (12/29), histopathology alone in 31% (9/29), and the combination of culture and histopathology in 28% (8/29). Ninety-three percent (14/15) of mucormycetes isolated from haematology patients were associated with probable/proven-IFD, compared with only 26% (6/23) in patients with chronic respiratory disease ($p<0.01$). Thirty eight percent (5/13) of trauma/burns/plastics patients were diagnosed with proven/probable-IFD. In all lung transplant patients (6/6) with colonization/no-IFD that had a mucormycete identified from a respiratory specimen, no endobronchial lesions were noted on bronchoscopy.

Proven/probable-IFD was compared with no-IFD/colonisation with regards to clinical features and risk factors (Table 2). On univariate analysis, patients with proven/probable IFD were significantly more likely to have a history of malignancy (52% vs. 16% $p<0.01$) and had an allogeneic SCT (28% vs. 0% $p<0.01$) (Table 2). Reflecting underlying risk, a higher number of proven/probable IFD patients were on antifungal prophylaxis/treatment (52%, 15/29) posaconazole, fluconazole, voriconazole, or liposomal amphotericin B) at the time of diagnosis compared with 14% (5/37) with no-IFD/colonisation ($p<0.01$) (Table 2). Twenty-four percent (7/29) of proven/probable IFD were receiving posaconazole prophylaxis (mean trough level 1.03mg/L, n=5) compared with 2.7% (1/37) of no-IFD/colonization ($p=0.02$). Fifty nine percent (17/29) of patients with proven/probable IFD were on immunosuppressant medications (including corticosteroids) compared with only 24% (9/37) of cases with no-IFD/colonisation ($p<0.01$) (Table 2). Table 2 outlines additional risk factors which are associated with proven/probable IFD.

Treatment, outcomes and mortality rates were compared for proven/probable-IFD versus no-IFD/colonisation (Table 3). Ninety-three percent (27/29) of patients with proven/probable IFD received antifungal treatment whilst only 30% (11/37) of those with no-IFD/colonisation were treated ($p<0.01$) (Table 3). Twenty-three (79%) patients with proven/probable IFD received intravenous liposomal amphotericin B (Ambisome®) (5mg/kg dosing) (Table 3). Treatment of skin and soft tissue infections often required surgical intervention (93%; 13/14). All patients (4/4) where mucormycetes were isolated form sinuses underwent surgical debridement.

Seventy-two percent of patients with proven/probable-IFD (21/29) had no recurrence of disease whilst 92% (34/37) of those with no-IFD/colonisation had no further positive cultures (Table 3). Thirty-day overall mortality was significantly higher in the proven/probable-IFD cohort (24%; 7/29) as compared with those who had no-IFD/colonisation (3%; 1/37) ($p=0.02$).

Of the proven/probable IFD cohort that received treatment, 78% (21/27) had no recurrence and 22% (6/27) died whilst still an inpatient. Of the of no-IFD/colonisation patients that were treated (11/37 [30%]), 82% (9/11) had no recurrence and one patient died (9%) as an inpatient. The single mortality in the no-IFD/colonization was due to methicillin sensitive *Staphylococcus aureus* bacteraemia and biventricular heart failure. There were two cases with available autopsy results, none of which demonstrated any evidence of invasive mucormycosis.

Discussion

We describe the current clinical features, risk factors, treatment and outcomes in patients that were diagnosed with mucormycosis at Alfred Health. Alfred Health is a single center but encompasses the major surgical (e.g. trauma/burns) and medical populations (e.g. CF, haematology and solid organ transplantation) at–risk for mucormycosis. Fifty-six percent of patients diagnosed with mucormycosis did not have clinically significant disease, and subsequently did not fulfill the criteria for proven/probable IFD. As a result they were defined as having no-IFD or colonisation. These patients were more likely to isolate a mucormycete from sputum samples or bronchial washings (84%). This supports the hypothesis that a large group of patients can be colonised with mucormycetes without having invasive or deep tissue infection. Ambrosioni *et al,* similarly found that 65% of patients in their study were colonised and the site most commonly colonised were the sinuses and upper respiratory tract [9].

Only 30% of the colonised patients in our study who received antifungal treatment, 45% of those treated received therapy with agents that had no activity against mucormycetes. Despite this the overall outcomes in the no-IFD/colonisation group were good with 92% having no recurrence and 97% surviving. This implies that mucormycete isolation does not always require treatment and is not associated with a poor outcome; however, its isolation needs to be correlated with the clinical history and findings.

The receipt of mold active prophylaxis should not reduce clinician suspicion of mucormycosis in at-risk groups. This is highlighted by the fact that 24% of patients with proven/probable disease were receiving posaconazole prophylaxis at time of diagnosis, with appropriate prophylaxis trough levels (>0.7mg/L).

Our data also support aggressive treatment in those that have proven/probable-IFD as mortality in this group is clinically significant (24%). Interestingly, the study by Skiada etal [2] had a mortality rate of 47% in their proven/probable cohort which is substantially higher than our study detected. Possible reasons for this may be that their study had significantly larger numbers (230 patients) and more patients (44% vs. 23% [in our study]) had a hematological malignancy. Risk factors including immunosuppression, prednisolone use, malignancy and transplantation need to be considered as they remain as strongly associated with invasive disease in our study. Similar findings were, also demonstrated in previous studies [10]. Although these data were not captured in our cohort, cigarette smoking has been linked with *Rhizopus* and *Rhizomuco*r spp. isolation[11, 12]. As Rhizopus and Rhizomucor were commonly identified in our cohort, smoking status would be an interesting risk factor to prospectively analyze in the future. Patients were likely to have multiple risk factors which were possibly synergistic; however our study was not able to assess this. Haematology patients were a cohort that were particularly at risk for proven/probable-IFD. Of the 15 haematology patients in our study, 100% received treatment, 60% had no recurrence and 33% died as inpatients.

Our study demonstrated that the majority of specimens were pulmonary (71%) however skin and soft tissue infections were also common (21%). Treatment of skin and soft tissue infections often required surgical intervention (93%; 13/14) in addition to systemic antifungal treatment and previous studies have shown that this results in better outcomes [13].

There are a number of limitations to our study that should be considered, including its retrospective nature. Small numbers are likely to have impacted on our ability to

make statistical associations, an inherent issue when examining rare IFD. As our study was restricted to cases identified from positive microbiology or histopathology records we may have potentially missed the possible cases where clinical suspicion was high and empirical treatment was given. Despite stringent examination of cultures for laboratory contamination, it is possible that cases reflect contamination rather than colonisation. Whilst some patients were considered 'colonized only' during the study period, potentially patients may have developed invasive mucormycosis post follow-up, which was not captured by our single institution. We were unable to comment on the duration of treatment patients received as once patients were discharged from hospital, follow up records were incomplete.

Despite these limitations, our study describes the current epidemiology of mucormycetes infections in a large group of immunocompromised hosts at an Australian tertiary referral center, particularly in the setting of newer antifungal agents as prophylaxis. The rate of mucormcyete isolation is likely to continue to rise as diagnostic assays improve and immunocompromised hosts live longer. We demonstrate that the historical risk factors for mucormycosis infection remain, however that isolation of mucormycetes does not necessarily equate to invasive disease and patients therefore may not require aggressive antifungal therapy. Those with proven/probable IFD do warrant aggressive surgical and antifungal treatment as mortality remains significant.

Conclusion

Mucormycosis remains a relatively uncommon fungal infection. Microbiological isolation does not necessarily equate to invasive disease with over 50% of patients in our study potentially being colonized for the study period. Those with traditional risk factors (haematological malignancy or transplantation, corticosteroid use and immunosuppression) and those with proven/probable-IFD according to modified EORTC/MSG criteria should be managed as inpatients and treated aggressively as mortality remains substantial in this group. Close follow up of 'colonized' patients is still required considering the potential morbidity and mortality associated with the development of invasive mucormycosis. Further studies assessing the efficacy of antifungal treatment and duration required, are needed.

References

1. Roden, M.M., et al., *Epidemiology and Outcome of Zygomycosis: A Review of 929 Reported Cases.* Clinical Infectious Diseases, 2005. **41**(5): p. 634-653.
2. Skiada, A., et al., *Original Article: 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.* Clinical Microbiology and Infection, 2011. **17**: p. 1859-1867.
3. Prabhu, R.M. and R. Patel, *Mucormycosis and entomophthoramycosis: a review of the clinical manifestations, diagnosis and treatment.* Clin Microbiol Infect, 2004. **10 Suppl 1**: p. 31-47.
4. Rogers, T.R., *Treatment of zygomycosis: current and new options*.
5. Lau, A., 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**(2): p. 380-5.
6. De Pauw, B., et al., *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.* Clinical infectious diseases : an official publication of the Infectious Diseases Society of America, 2008. **46**(12): p. 1813-1821.
7. Slavin, M., et al., *Invasive infections due to filamentous fungi other than Aspergillus: epidemiology and determinants of mortality.* Clin Microbiol Infect, 2015.
8. Husain, S., et al., *A 2010 working formulation for the standardization of definitions of infections in cardiothoracic transplant recipients.* J Heart Lung Transplant, 2011. **30**(4): p. 361-74.
9. Ambrosioni, J., K. Bouchuiguir-Wafa, and J. Garbino, *Emerging invasive zygomycosis in a tertiary care center: epidemiology and associated risk factors.* International Journal of Infectious Diseases, 2010. **14**(Supplement 3): p. e100-e103.
10. Fleming, S., et al., *Consensus guidelines for antifungal prophylaxis in haematological malignancy and haemopoietic stem cell transplantation, 2014.* Intern Med J, 2014. **44**(12b): p. 1283-97.
11. Gomes, M.Z., R.E. Lewis, and D.P. Kontoyiannis, *Mucormycosis caused by unusual mucormycetes, non-Rhizopus, -Mucor, and -Lichtheimia species.* Clin Microbiol Rev, 2011. **24**(2): p. 411-45.
12. Ogundero, V.W. and J.O. Aina, *Storage temperature and viability of sporangiospores of potentially human pathogenic species of Rhizomucor from Nigerian tobacco.* J Basic Microbiol, 1989. **29**(3): p. 171-5.
13. Meis, J.F. and A. Chakrabarti, *Changing epidemiology of an emerging infection: zygomycosis.* Clinical Microbiology & Infection, 2009. **15**: p. 10-14.

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Table 1: Baseline characteristics for the cohort as per proven, probable or no-invasive fungal disease/colonisation

	Proven/ Probable (n=29) (n, %)	Colonisation (n=37) (n, %)	Total (n=66) (n, %)
Age (Median)	55 years	58 years	56.5 years
Male	19 (66)	29 (78)	48 (73)
Rural residence	13 (45)	11 (30)	24 (36)
Unit			
- Outpatient	3 (10)	12 (32)	15 (23)
- Inpatient	26 (90)	25 (68)	51 (77)
- Haematology	14 (48)	1 (3)	15 (23)
- Respiratory	3 (10)	9 (24)	12 (18)
- Trauma/Burns/Plastics	5 (17)	8 (22)	13 (20)
- Other[a]	4 (14)	7 (19)	11 (17)
Site of isolation			
- Lung/Bronchus	16 (55)	31 (84)	47 (71)
- Skin and soft tissue	8 (28)	6 (16)	14 (21)
- Sinus	4 (14)	-	4 (6)
- Colon	1 (3)	-	1 (2)
Species			
- *Rhizopus spp*	17 (59)	15 (41)	32 (48)
- *Rhizomucor spp*	3 (10)	16 (43)	19 (29)
- *Mucor spp*	2 (7)	6 (16)	8 (12)
- *Mucormycosis (NOS)*	6 (21)	-	6 (9)
- *Saksenae vasiformis*	1 (3)	-	1 (2)
Diagnosis[b]			
- Histopathology	17 (59)	-	17 (26)
- Microbiology (culture)	20 (69)	37 (100)	57 (86)
- Panfungal PCR	6 (21)	0 (0)	6 (9)
Season at diagnosis			
- Summer/Spring	17 (59)	20 (54)	37 (56)
- Winter/Autumn	12 (41)	17 (46)	29 (44)

Abbreviations: NOS, not otherwise specified

[a] Cardiology (N=2), Gastroenterology (N=1), General Medicine (N=3), Infectious Diseases (N=1), Neurosurgery (N=1), Renal (N=1), Rheum (N=1), Vascular Surgery (N=1).

[b] Histopathology, Microbiology (culture) or panfungal PCR positive.

Table 2: Clinical factors associated with proven/probable IFD /compared with No IFD (colonisation)

	Proven/ probable IFD n=29 n (%)	Colonisation n=37 n (%)	P value
Diabetes	10 (34)	9 (24)	0.42
Transplant	15 (52)	7 (19)	<0.01
- Haematological[a]	8 (28)	-	<0.01
- Lung	5 (17)	6 (16)	1.0
- Heart	1 (3)	1 (3)	1.0
- Heart/lung	1 (3)	-	0.44
Malignancy	15 (52)	6 (16)	<0.01
- Haematological	14 (48)	1 (2)	<0.01
- Other[b]	1 (3)	5 (14)	0.22
Immunosuppressant medications	17 (59)	9 (24)	<0.01
- Prednisolone >20mg/day	7 (24)	2 (5)	0.04
Antifungal prophylaxis/treatment[c]	15 (52)	5 (14)	<0.01
- Posaconazole[d]	7 (24)	1 (3)	0.02
- Fluconazole	1 (3)	2 (5)	1.0
- Voriconazole[e]	5 (17)	1 (3)	0.08
- Liposomal Amphotericin B	2 (7)	0	0.19
- Itraconazole	0	1 (3)	1.0
Rural address	13 (45)	11 (30)	0.30

[a] All 8 stem cell transplant (SCT) patients received allogeneic transplants.

[b] Malignancy types: Lung cancer (N=2), squamous cell carcinoma (N=2), melanoma (N=1), colon

carcinoma (N-1).

[c] Patients receiving antifungal therapy at time of mucormycete isolation

[d] Posaconazole trough level in 5 patients (mean 1.03mg/L, Range: 0.264 – 2.18mg/L)

[e] Voriconazole trough level in 4 patients (mean 2.85mg/L, Range: 1.1-4.6mg/L)

Table 3: Antifungal therapy, outcomes and mortality in proven/probable- IFD versus no-IFD/colonisation

	Proven/ probable IFD n=29	Colonisation n=37	P value
Antifungal Therapy			
- No treatment	2* (7)	26 (70)	<0.01
- Antifungal treatment	27 (93)	11 (30)	<0.01
- Liposomal amphotericin B	23 (79)	4 (11)	<0.01
- Posaconazole	3 (10)	2 (5)	0.65
- Fluconazole[a]	1 (3)	2 (5)	1.00
- Other[b]	0 (0)	3 (8)	0.62
- Surgery	21 (72)	6 (16)	0.001
Outcome			
- No recurrence	21 (72)	34 (92)	0.05
- Inpatient death	7 (24)	1 (3)	0.02
- 30 day mortality	7 (24)	1 (3)	0.02
- 60 day mortality	7 (24)	1 (3)	0.02
- Unknown outcome	1 (3)	-	-

*1 patient died prior to having the opportunity to receive treatment

[a] Patients receiving fluconazole post mucormycete isolation received prophylaxis dosing and there were no proven cases of mucormycosis.

[b] Caspofungin (N=1), itraconazole (N=1), voriconazole (N=1).