The distribution, patient characteristics, therapy and patient outcome in culture positive invasive mold infections in a tertiary hospital in the Free State province, South Africa.

Submitted in fulfilment of the requirements in respect of the Master's Degree MMed in the Department of medical microbiology in the Faculty of health sciences at the University of the Free State.

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Declaration of authorship:

I, Dr B van der Westhuizen, declare that the coursework Master's Degree mini-dissertation that I herewith submit in a publishable manuscript format for the Master's Degree qualification MMed in the Department of medical microbiology in the Faculty of health sciences at the University of the Free State is my independent work, and that I have not previously submitted it for a qualification at another institution of higher education.

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The distribution, patient characteristics, therapy and patient outcome in culture positive invasive mold infections in a tertiary hospital in the Free State province, South Africa.

Key words:
Invasive mold infection, mold distribution, risk factors, treatment, outcome

List of abbreviations:
UAH – Universitas Academic Hospital, SANAS – South African National Accreditation System, NICD - National Institute of Communicable Diseases, NHLS – National Health Laboratory Services, HIV – Human Immunodeficiency Virus, IRIS – Immune Reconstitution Inflammatory Syndrome, ICU – Intensive Care Unit, STATS SA – Statistics SA, ABD – Amphotericin B deoxycholate, PD – Peritoneal Dialysis

Word count:
Abstract - 286
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Abstract
Introduction
Fungi, including molds, are increasingly recognized as important pathogens carrying a high morbidity and mortality in critically ill and immune compromised patients and our understanding of these diseases remain incomplete, largely due to the lack of surveillance data. This study aimed to better quantify the distribution, patient characteristics, risk factors, therapy and treatment outcome in culture positive invasive mold infections at Universitas Academic Hospital in the Free State province, South Africa.

Methods
All culture positive mold isolates cultured from sterile specimens were identified retrospectively from 1 July 2014 to 30 June 2017. Laboratory and clinical data were reviewed for those that met the inclusion criteria.

Results
A total of 48 isolates were included in this study. There was a similar distribution between males and females and the mean age was 40.5 years. *Aspergillus* species were the most commonly isolated mold. The most common risk factors identified were HIV infection with a median CD4 of 88.5 cells/µl followed by hematological conditions. The treatment strategies in our study group were heterogeneous with 73.1% (19/26) of patients treated with antifungal therapy alone, 19.2% (5/26) with surgery alone and 7.7% (2/26) with a combined medical and surgical approach. Many patients received no treatment 45.8% (22/48). The overall mortality was 25% (12/48).

Conclusions
The diagnosis of invasive mold infections remains a challenge. In the current study, molds were found to cause serious infections, especially in at risk patients. Despite treatment with appropriate antifungal

agents, the associated mortality rate was still high. This study contributes to the growing knowledge on the distribution, patient characteristics and outcomes of invasive mold infections, particularly in patients in the Free State, and lays the foundation for further research in the field of invasive mold infections.

A case series: The distribution, patient characteristics, therapy and patient outcome in culture positive invasive mold infections in HIV positive patients in a tertiary hospital in the Free State province, South Africa

Key words:

HIV, CD4, Invasive mold infection, mold distribution, treatment, outcome

List of abbreviations:

UAH – Universitas Academic Hospital, HIV – Human Immunodeficiency Virus, IRIS – Immune Reconstitution Inflammatory Syndrome, ICU – Intensive Care Unit, STATS SA – Statistics SA, ABD – Amphotericin B deoxycholate

Word count:

Abstract - 238
Article - 2194

Abstract

Introduction

Fungi, including molds, are increasingly recognized as important pathogens carrying a high morbidity and mortality in critically ill and immune compromised patients and our understanding of these diseases remain incomplete, largely due to the lack of surveillance data. This study aimed to better quantify the distribution, patient characteristics, risk factors, therapy and treatment outcome in culture positive invasive mold infections at Universitas Academic Hospital (UAH) in the Free State province, South Africa. This case series describes the HIV positive patients in this study.

Methods

All culture positive mold isolates cultured from sterile specimens were identified retrospectively from 1 July 2014 to 30 June 2017. Laboratory and clinical data were reviewed for those that met the inclusion criteria.

Results

The most common risk factors identified were HIV infection with a median CD4 of 88.5 cells/µl and hematological conditions. *Sporothrix schenckii* and *Bipolaris* species were the most common molds identified in the HIV positive patients. The documented mortality was (2/14)14.2%.

Conclusions

HIV infection is a common risk factor for invasive mold infections in South Africa. Advanced disease did not equate to mortality and the majority of patients responded well to appropriate therapy. The importance of source control was also demonstrated. This study contributes to the growing knowledge on the distribution, patient characteristics and outcomes of invasive mold infections, particularly in patients in the Free State, and lays the foundation for further research in the field of invasive mold infections.

A case series: The distribution, patient characteristics, therapy and patient outcome in culture positive invasive mold infections in patients with hematological conditions in a tertiary hospital in the Free State province, South Africa

Key words:

Invasive mold infection, treatment, outcome, hematological conditions, neutropenic

List of abbreviations:

UAH – Universitas Academic Hospital, HIV – Human Immunodeficiency Virus, ICU – Intensive Care Unit, ABD – Amphotericin B deoxycholate, IFIs – Invasive Fungal Infections, AML – Acute Myeloid Leukemia, ALL – Acute Lymphocytic Leukemia

Word count:

Abstract - 250
Article - 1850

Abstract

Introduction

Fungi, including molds, are increasingly recognized as important pathogens carrying a high morbidity and mortality in critically ill and immune compromised patients and our understanding of these diseases remain incomplete, largely due to the lack of surveillance data. This study aimed to better quantify the distribution, patient characteristics, risk factors, therapy and treatment outcome in culture positive invasive mold infections at Universitas Academic Hospital (UAH) in the Free State province, South Africa. This case series describes the patients with underlying hematological conditions in this study.

Methods

All culture positive mold isolates cultured from sterile specimens were identified retrospectively from 1 July 2014 to 30 June 2017. Laboratory and clinical data were reviewed for those that met the inclusion criteria.

Results

We identified hematological conditions to be the second most common risk factor in patients with culture positive mold infections only second to HIV. There were very few patients with a hematological condition as well as HIV as underlying risk factors 16.2% (2/12). The most common mold species were *Aspergillus* species followed by the mucoraceous molds. The documented mortality was 41.7% (5/12) in the patients affected by hematological conditions.

Conclusions

IFIs are a major cause of morbidity and mortality in patients affected by hematological disorders, especially in the setting of neutropenia. This study contributes to the growing knowledge on the distribution, patient characteristics and outcomes of invasive mold infections, particularly in patients in the Free State, and lays the foundation for further research in the field of invasive mold infections.

Chapter 1

Literature review

Invasive fungal infections (IFIs) are important causes of morbidity and mortality. IFIs have assumed a much greater importance in recent years. Mold infections, in particular, have become more common [1, 2]. This is mainly because of the increasing size of the population at risk. The most common causes of IFIs are *Candida albicans* and *Aspergillus* species. Different species are reported from different institutions. Other fungi that have increasingly been associated with infections in recent years include non-albicans *Candida* species, *Cryptococcus* and *Trichosporon,* molds such as *Fusarium* and the Zygomycetes. *Aspergillus fumigatus* is the most common cause of aspergillosis. Other *Aspergillus* species are less commonly encountered. Although an increase in aspergillosis is observed, there is a paucity of data on the proportions of infections caused by the different Aspergillus species [2].

Candida species and *Cryptococcus* species have been identified as the most common causes of invasive yeast infections. These two fungi have been studied extensively, both internationally and locally. The most common molds isolated in international studies are *Aspergillus* species, *Fusarium* species and *Rhizopus* species [3, 4, 6, 7]. In contrast, there is limited local data, probably due to diagnostic challenges, on invasive mold infections, with these species. A recently published article based on results from Kwazulu-Natal, a province in South Africa, demonstrated that *Aspergillus* species were the most commonly isolated mold species among critically ill children [8]. More data have also become available about *Emergomyces africanum*, in South Africa, in the last few years. IFIs related to HIV infection are endemic to the Western Cape in South Africa and the most common species presenting with skin lesions identified in a study published in 2017 is *Emergomyces africanum*, *Histoplasma capsulatum* and *Sporothrix schenckii* [9]. Other research done in South Africa, has also shown an increase in patients presenting with an unmasking IRIS with *Emergomyces africanum* (formerly known as *Emmonsia* species) [10, 11].

Disseminated histoplasmosis is a known AIDS-defining disease that was classified as such in 1987 [12]. In 2013, Armstrong-James, D et al reported that roughly 25% of patients with stage 4 HIV-1 infection presents with this infection in endemic areas. *Histoplasma duboisi* have also became an important pathogen in patients with advanced HIV infection [13]. The biggest risk factor for histoplasmosis is the spread of HIV, although the use of immunosuppressive agents also contributes to this increase [12].

Chronic pulmonary aspergillosis is a common complication, increasingly seen, due to structural lung damage secondary to tuberculosis. Previously, pulmonary aspergillosis was mostly observed in patients who were neutropenic secondary to zidovudine therapy, but cases may also be seen in patients with advanced HIV infection [4]. It was recently estimated that the proportion of patients with chronic pulmonary aspergillosis in South Africa is at 175.8/100 000.This number is probably one of the highest in the world and it may partially be attributed to South Africa’s high HIV infection and TB rate [14]. It is also a well-known fact that invasive aspergillosis is associated with hematological conditions [15].

Penicilliosis is also a common AIDS-defining infection in endemic Southeast Asia [4]. There is a paucity of local data on this particular mold.

Sporothrix schenckii infection is usually associated with individuals working in the agricultural sector and therefore mostly males. This is presumably due to the fact that traumatic inoculation is the most common route of infection. However, this mold may infect patients of all ages and genders. *Sporothrix schenkii* is associated with disseminated infection in patients with advanced HIV [16, 17].

A systematic review, including 61 articles, on mucormycosis in HIV infected patients published in 2016, have found that mucormycosis is a serious infection in HIV infected individuals. This infection carries a very high mortality and is seen especially in patients also infected with other HIV-associated opportunistic infections and in those with very low CD4 cell counts. Other important risk factors are diabetes mellitus, intravenous drug use, neutropenia and corticosteroid use [18].

Risk factors for developing IFIs include prolonged ICU stay, solid organ transplants, hematopoietic stem cell transplants, hematological malignancies, chemotherapy, immunosuppressive medication, neutropenia, burn wounds, HIV infection, invasive medical devices and grafts. Other important risk factors include low birth weight neonates and total parenteral nutrition. Broad spectrum antibiotics and more aggressive surgery have also been identified as important contributing factors [2, 4, 6, 13].

Although it is a well-known fact that invasive aspergillosis is associated with hematological conditions, studies have shown that there is in increase in infections with molds like the Zygomycetes, *Fusarium* species and *Bipolaris* species [15]. There are many different hematological conditions that can affect patients and the risk of developing systemic mycoses may be different for the different conditions. These risk factors need to be thoroughly evaluated as this may lead to inappropriate therapy in many patients resulting in over- or under treatment [19]. It has been documented that IFIs are on the increase compared to invasive yeast infections that are decreasing [19]. There are many possible challenges contributing to this change in epidemiology in patients affected by hematological conditions including selective pressure of antifungal prophylaxis, high numbers of high risk patients, low thresholds to start empiric therapy and limitations of currently available diagnostics [20]. Patients with an exceptionally high risk for developing IFIs are patients with acute myelocytic leukemia and allogeneic hematopoietic stem cell transplant recipients. There are also additional conditions that predisposes these individuals to develop IFIs including neutropenia, relapse disease, refractory disease, previous history of IFIs and therapy with immunosuppressive agents [19].

The diagnosis of IFIs has proven to be very difficult, posing a diagnostic dilemma, as the clinical manifestations are not specific and the majority of available tests lack sensitivity. Due to these diagnostic challenges it is difficult to determine the true burden of disease due to these pathogens [7]. Culture has a suboptimal sensitivity, ranging from 8-62% for *Aspergillus* species and <25% for the mucoraceous molds, and may require incubation for several days to weeks before growth is observed [21]. The sensitivity for culture from a sputum sample for acute pulmonary histoplasmosis is 10-15%, however, Wright-Giemsa stained peripheral blood smears are positive in up to 40% of these patients [12].A

histological diagnosis is often not practical and the sensitivity of a histopathological examination for the identification of fungal elements is roughly 78% [22]. Alternatives to culture based methods, have been developed in an attempt to diagnose IFIs earlier. The use of 1, 3-Beta-D-Glucan has proven to be useful in diagnosing common invasive fungal infections in at risk patients, and the serial quantification of galactomannan has proven to be useful in the diagnosis of suspected *Aspergillus* infection. The pooled sensitivity of 1, 3-Beta-D-Glucan is around 76.8% and the specificity around 85.3%. 1,3-Beta-D-Glucan testing offers a much better sensitivity in comparison to culture, however, it is expensive, labour intensive and not widely available in most developing countries. 1, 3-Beta-D-Glucan testing is mainly helpful in the setting of its high negative predictive value and can be used to rule out invasive IFIs and withhold antifungal therapy but there are many causes (including antibiotics frequently used it this patient population) of false positive results [23]. The serum galactomannan test is very useful for neutropenic patients. This is largely due to its high negative predictive value (>98%).A positive serum galactomannan result in patients who are not neutropenic is of limited value due to its suboptimal sensitivity, however, it's detection in bronchoalveolar lavage specimens is very useful in non-neutropenic hosts [23]. PCR-based methods' major disadvantage is that it's availability is currently limited to reference mycology laboratories, however, it should be considered as a diagnostic tool when available [24]. A urine antigen test is available for the diagnosis of histoplasmosis. This test has recently been introduced at by the Mycology Reference Laboratory at NICD-NHLS. The test is still being validated and requires a urine specimen that has been refrigerated after collection [12]. With regards to aspergillosis, the most important laboratory test, IgA antibody, is not available in RSA [14].

Contamination of clinical samples with molds can occur any time from specimen collection to laboratory processing. Molds are one of the most common contaminants encountered in microbiology. Molds are environmental organisms and the spores of most molds are very light and easily transported by air. Therefore, just neglecting to clean a skin lesion properly before biopsy or just leaving the lid of a petri dish open for a few seconds, allows for the entry of contaminating organisms. The majority of contamination occurs through avoidable procedural errors. Proper specimen collection and correct laboratory practices is of utmost importance if an invasive mold infection is suspected. One of the biggest issues confronting the cultivation of a mold is contamination by other molds [25, 56].

Definitions for IFI's for clinical and epidemiological research were published in 2002; these definitions were revised in 2008. These definitions include proven, probable and possible IFIs. Proven fungal infections are required to fulfill a mycological criterion from a sterile clinical sample (histology/culture). Probable IFIs are required to fulfill a mycological criterion from a non-sterile clinical sample, a host criterion and a clinical criterion. Possible IFIs include only those cases with risk factors, clinical signs and symptoms consistent with IFIs but without mycological support [27].

The development of new and effective antifungal treatment is hampered by the fact that fungi are eukaryotic organisms and therefore drug development is limited by the lack of fungal-specific drug targets [13]. In a resource limited country like South Africa, the most commonly used drugs to treat IFIs are amphotericin B (ABD) and fluconazole. ABD has a broad spectrum of activity, but side effects are

common, occurring in more than half of the patients. Side effects are mainly nephrotoxicity or infusion related events. Other side effects include hypokalemia, fevers, nausea, vomiting and hypotension. Nephrotoxicity is aggravated by the use of other drugs that affects renal function and diuretics, that are often required in the population at risk, as well as the dosage and the duration of treatment required [4, 28]. Liposomal ABD is less nephrotoxic, but this formulation is expensive and not widely available [28]. Fluconazole is well tolerated, with few drug interactions, but is inactive against molds. However, it is often used as prophylaxis in hematology units to prevent invasive yeast infections [1, 28]. The newer azoles and echinocandins have a wider spectrum of activity, which includes selected molds. Therefore, most patients in South Africa are treated with prolonged courses of ABD with its associated side effects [7].

The patterns in antifungal resistance has also been changing [2, 3]. Advances made in clinical medicine have led to changes in the incidence and drug resistance of fungal infections. This includes the practice where ABD is the main drug used for empiric therapy against IFIs in most centers and azoles are used more frequently for antifungal prophylaxis in at risk patients. A shift towards infections with azole and ABD resistant fungi has been reported, which may be associated with especially poor outcomes [2, 3].

Despite general agreement that invasive fungal diseases are becoming more important, our understanding of these diseases remain incomplete, mainly due to the lack of surveillance data. Analysis of death records in the USA showed that fungal infections were the 7th most common cause of death due to infections in 1992, and that mortality secondary to IFIs has increased significantly since 1980. Candidiasis and aspergillosis accounted for most of these deaths [6].

In this context, it was proposed in 2011, that the following fungal diseases be added to the list of Neglected Tropical Diseases (NTD's): Cryptococcal meningitis, *Pneumocystis jiroveci* pneumonia, Mycetoma, Histoplasmosis, Sporotrichosis and Blastomycosis. This would help to determine the epidemiology and burden of disease. This information will also allow the development of strategies to control and prevent of these neglected fungal diseases [7]. In May 2016 mycetoma was acknowledged as a neglected tropical disease [29].

Fungiscope is a Global Emerging Fungal Infection Registry that was established in 2003 to improve knowledge on epidemiology, clinical manifestations and treatment strategies for invasive fungal infections [30].

There is very limited published data on the distribution, treatment and patient outcomes of IFIs in South Africa, and the Free State in particular.

2. Aim

A retrospective data collection on the distribution, patient characteristics, therapy and patient outcomes in culture positive invasive mold infections at Universitas Hospital in the Free State province, South Africa.

3. Objectives

- To determine the spectrum of invasive mold infection pathogens in patients with culture positive invasive mold infections.
- To determine the baseline characteristics of patients with culture positive invasive mold infections.
- To assess the treatment outcome in these patients.

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The distribution, patient characteristics, therapy and patient outcome in culture positive invasive mold infections in a tertiary hospital in the Free State province, South Africa.

Word count - 3471

Introduction

Fungi, including molds, are increasingly recognized as important pathogens in critically ill and immune compromised patients [1, 2]. Not only have these organisms assumed a greater role in human disease over the last two decades, but invasive fungal infections, and in particular mold infections, are associated with significant morbidity and mortality [3, 4].

Despite general agreement that invasive fungal diseases are becoming more important, our understanding of these diseases remain incomplete, mainly due to the lack of surveillance data. The most common isolated molds in international studies are *Aspergillus* species, *Fusarium* species and mucoraceous molds [1, 5, 6, 7]. A recently published article from Kwazulu-Natal, South Africa reported that *Aspergillus* species was the most commonly isolated mold species amongst critically ill children [8]. Recent studies done in South Africa reported an increase in HIV positive patients presenting with an unmasking IRIS with *Emergomyces africanum* (formerly known as *Emmonsia* species) [9, 10]. IFIs associated with HIV infection is endemic to the Western Cape province in South Africa with the most common species presenting with skin lesions identified in a study published in 2017 being *Emergomyces africanum*, *Histoplasma capsulatum* and *Sporothrix schenckii* [11]. Except for these publications, there is limited local data, regarding invasive mold infections, most likely due to diagnostic challenges.

With a retrospective review of laboratory and patient data, this study aimed to better quantify the distribution, patient characteristics, risk factors, therapy and treatment outcome in culture positive invasive mold infections at Universitas Academic Hospital (UAH) in the Free State province, South Africa.

Methods

Study design and setting

A retrospective, observational descriptive study was performed. Patients admitted to UAH between 1 July 2014 and 30 June 2017, in whom a mold was isolated from fungal culture, were included in the study. UAH is the only tertiary referral hospital providing specialist and sub-specialist level care for the Free State and Northern Cape provinces.

All culture positive molds from sterile sites (tissue specimens, blood cultures, peritoneal fluid and cerebrospinal fluid) were included in the final analysis. Although bronchoalveolar lavage fluid, endotracheal aspirates and sputum specimens are not considered sterile specimens, they were still included in the study if the same mold was cultured from a second specimen, and the patient had symptoms of an invasive fungal disease of the lungs together with supporting clinical and/or radiological signs.

We excluded all specimens that were negative for fungal growth, specimens that cultured a yeast, specimens that cultured a mold from a non-sterile site (except for selected respiratory specimens) and non-human specimens.

Sample Analysis

Isolates were cultured at the Universitas National Health Laboratory Service Microbiology Laboratory, a SANAS (South African National Accreditation System) accredited laboratory. All specimens had been processed according to the standard operating procedure in the laboratory [12]. Specimens were inoculated onto 2 Sabouraud-Dextrose agar plates and incubated at 25 °C and 37 °C respectively for 14 days. The plates were examined daily for fungal growth. When growth was observed, the macroscopic appearance of the isolates was described, and a lacto-phenol-cotton-blue stain was performed for the microscopic examination to determine the identity of the mold. The molds that proved difficult to identify in our laboratory, were sent to the mycology reference laboratory at the NICD (National Institute of Communicable Diseases) for broad-range fungal polymerase-chain-reaction.

Data Collection

Laboratory results were obtained from the Central Data Warehouse at the NICD. All specimens registered for mycology culture from UAH for the period 1 July 2014 to 30 June 2017 were reviewed. Patient information was collected from the electronic patient file system, Meditech, used at UAH.

Patient clinic and hospital files were used when the electronic information was not available. Patients for whom no clinical data was available were excluded from the study. Data was captured with the help of an Infectious Diseases Physician, on a Microsoft Excel spreadsheet approved by the department of biostatistics of the University of the Free State.

Data Analysis

Descriptive statistics namely means and standard deviations or medians and percentiles, were calculated for continuous data. Frequencies and percentages were calculated for categorical data. The analysis was performed by the Department of Biostatistics.

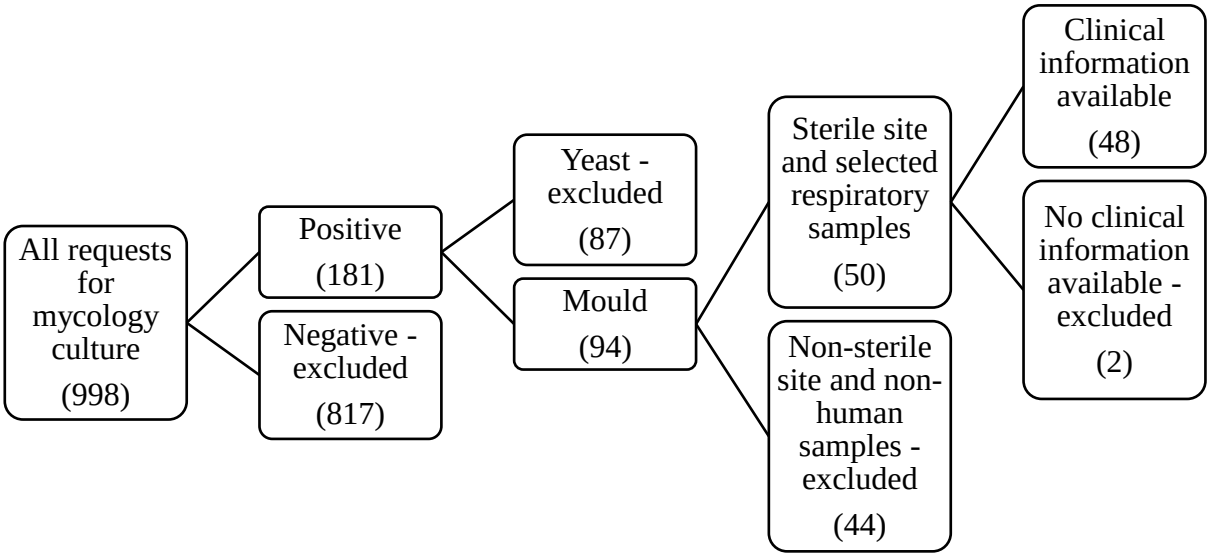
Ethical aspects

Approval to conduct the study was obtained from the NHLS business manager and acting Head of Department for Medical Microbiology as well as the Free State Department of Health, the Health Sciences Research Ethics Committee of the University of the Free State (HSREC 110/2017, UFS-HSD2017/1122). Confidentiality was ensured by allocating a number to each patient's record and by excluding all patients' personal information.

Results

From a total of 998 samples submitted for mycology culture, 950 were excluded based on the above described exclusion criteria as depicted in figure 1.

Figure 1. Patient selection



The remaining 48 samples were followed up and the results are depicted in Tables below.

Table 1. Summary of specimen type

Outcome	**Number of specimens**	**Percentage of specimens**
Specimen type	**Sample size 48**	**%**
Respiratory specimens	8	16.7
Tissue	29	60.4
Blood culture	1	2.1
Peritoneal fluid	8	16.7
Cerebrospinal fluid	2	4.2

Table 1 summarizes specimen distribution of the culture positive samples. Tissue was the most common sample type from which a mold was isolated, followed by respiratory samples and peritoneal dialysis fluid.

The presence of invasive fungal infection was confirmed upon histological examination in 37.9% (11/29) tissue samples from which a mold was cultured. In 20.7% (6/29) of the tissue specimens, histological evaluation was not requested.

It is noteworthy, that although the tissue samples that were negative for fungal growth were not included in this study for further analysis, roughly 70% of these were also submitted for histological examination. Upon histological examination 40% of these tissue samples were negative for fungal elements, approximately 10% were positive for fungal elements and the rest were suggestive of other pathology, namely malignancies, syphilis and drug reactions. Less frequently suggestive diagnoses included *Mycobacterium tuberculosis*, Mycobacteria other than *Mycobacterium tuberculosis*, *Echinococcus granulosis* and various other skin conditions.

Table 2. Baseline characteristics of the patients

Outcome	**Number of patients**	**Percentage of patients**
Sex	**Sample size 48**	**%**
Male	24	50
Female	24	50
Risk factor		
HIV	14	29.2
Hematological malignancy	9	18.8
Neutropenia	8	16.7
Chemotherapy	8	16.7
Chronic kidney disease on Peritoneal dialysis	8	16.7
Solid organ malignancy	3	6.3
Structural lung disease	3	6.3
Diabetes mellitus	2	4.2
Aplastic anemia	2	4.2

Allergic rhinosinusitis with nasal polyps	2	4.2
Steroid therapy	1	2.1
Primary immunodeficiency	1	2.1
Myeloproliferative disease	1	2.1
Ventriculo-peritoneal shunt	1	2.1
None identified	5	10.4

Table 2 summarizes the baseline patient characteristics. Males and females had equal numbers of positive samples and the median age was 40.5 years (range 7-78 years). The most common risk factor was HIV with only 10.4% (5/48) of patients having no identifiable risk factors. The median CD4 count in the HIV positive patients was 88.5 cells/µl (range 1-568 cells/µl). There were no patients that received bone marrow or solid organ transplants and no patients fulfilled the criteria for prolonged ICU stay defined as > 7 days.

Table 3. Mold species isolated

Outcome	**Number of specimens**	**Percentage of specimens**
Species isolated	**Sample size 48**	**%**
Aspergillus species	19	39.6
Fusarium species	5	10.4
Bipolaris species	5	10.4
Mucoraceous molds	4	8.3
Cladosporium species	3	6.3
Sporothrix schenckii	3	6.3
Penicillium species	2	4.2
Alternaria species	2	4.2
Histoplasma capsulatum	1	2.1
Neurospora species	1	2.1

Chaetomium species	1	2.1
Phoma species	1	2.1
Emmonsia species	1	2.1

Table 3 describes the mold distribution of culture positive isolates. *Aspergillus* species was by far the most commonly isolated mold followed by *Fusarium* species, *Bipolaris* species and the mucoraceous molds.

Table 4. Summary of treatment results

Outcome	**Number of patients**	**Percentage of patients**
Treatment (antifungal agent/s and/or surgery)	**Sample size 48**	**%**
Yes	26	54.2
No	22	45.8
Treatment modality		
Antifungal therapy alone	19	39.6
Surgery alone	5	10.4
Surgery plus antifungal therapy	2	4.2
Antifungal agents		
ABD	17	35.4
Fluconazole	7	14.6
Itraconazole	7	14.6
Voriconazole	5	10.4
Terbinafine	1	2.1

Table 4 summarizes the treatment modalities chosen for each patient. The majority of patients were treated with antifungal therapy alone and of these 78.9% (15/19) received combination therapy, mostly with ABD (Amphotericin B deoxycholate) combined with an azole. Only 26.9% (7/26) of patients received surgical intervention, with 2 receiving additional antifungal therapy.

Table 5. Outcome

Outcome	**Number of patients**	**Percentage of patients**
Patient response	**Sample size 48**	**%**
Improved	32	66.7
Unchanged	1	2.1
Demised in hospital	12	25
Unknown	3	6.3

Table 5 describes the patient outcome in our study. Most of the patients had a favorable outcome but the in-hospital mortality was 25%. The mortality rate amongst the treated patients was 23.1% (6/26) and 27.3% (6/22) in the untreated group (p-value 0.31).

Discussion

Diagnosis of invasive fungal infections remains a challenge, as the clinical manifestations are often non-specific. A lack of reliable diagnostic testing makes it difficult to estimate the true burden of fungal disease [6]. The reported sensitivity of histopathological methods for diagnosing invasive fungal infections is approximately 78%, as compared to 8-60% for culture [13, 14].

In our study 37.9% (11/29) had positive histology, but 20.7% (6/29) of tissue samples collected were not submitted for histological examination, thus probably underestimating the true sensitivity of histology. Amongst the culture negative samples, a large number of those submitted for histology identified another pathology and some identified an invasive mold infection. Although the sensitivity of histological identification of invasive mold infection in our study was low, it is clear that histology is helpful in diagnosing additional invasive fungal infections that may have been missed by culture as well as for diagnosing other infectious and non-infectious conditions. This underscores the need for closer interaction between the laboratory and clinicians regarding the submission of appropriate specimens, especially for difficult to diagnose infections.

In keeping with the findings of studies performed internationally, we also found that *Aspergillus* species followed by *Fusarium* species, *Bipolaris* species and the mucoraceous molds were the most common fungi isolated [1, 5, 6, 7]. Enoch *et al* reported that the most common mold isolated in the UK is *Aspergillus* species, although *Fusarium* species, *Scedosporium* species, *Penicillium* species and the Zygomycetes are increasingly seen [1]. Malani *et al* reported a similar picture in the USA [7].

Risk factors for developing invasive fungal infections include prolonged ICU stay, solid organ transplants, hematopoietic stem cell transplants, hematological malignancies, neutropenia, burn wounds, HIV infection, invasive medical devices and grafts. The use of antineoplastic and immunosuppressive agents, broad spectrum antibiotics and more aggressive surgery have also been identified as important contributing factors [1, 4, 5, 15]. The risk factors identified in our study population generally reflect those reported by others, except for HIV that is the most common risk factor in our study. The risk factors identified in international studies are diverse and in most patients multiple, however, hematopoietic stem cell transplant recipients, patients with hematological malignancies and neutropenia are described as the most common risk factors. A large multicenter trial done in Asia identified prolonged corticosteroid use as the most common risk factor to develop IFI's in their setting [16].

We identified HIV as the most common risk factor (median CD4 88.5 cells/µl), a finding that has previously not been well documented in the literature. This may be due to the paucity of data from countries with a high burden of HIV infection. It should be noted that the background prevalence of HIV in the Free State is estimated at 5.1% as released in the midyear population estimates for 2018 by STATS SA (Statistics South Africa) [17]. The mold species isolated from the 14 HIV positive patients were *Sporothrix schenkii*, *Bipolaris* species, *Aspergillus* species all at 21.4% (3/14), *Histoplasma capsulatum*, *Emmonsia* species, *Penicillium* species, *Cladosporium* species all at 7.1% (1/14). Interestingly a tissue sample from 1 patient with rhino-orbital-cerebral mucormycosis isolated a *Saksenaea oblongispora*, confirmed by PCR. This organism has recently been recognized as an emerging Zygomycete [18]. To our knowledge, this is the first case of invasive *Saksanaea oblongispora* infection described in the setting of HIV.

The most common mold species isolated from the 12 patients with hematological conditions were *Aspergillus* species 33.3% (4/12), which is in keeping with that reported in the literature. Studies have also shown that there is in increase in infections with Zygomycetes, *Fusarium* species and *Bipolaris* species [19]. This is in keeping with our findings with the mucoraceous molds being the second most common fungal isolate in this patient group. The majority of these patients with hematological conditions were neutropenic at 66.7% (8/12) at the time of culture collection and 16.2% (2/12) were known to be HIV positive.

There are many barriers to the development of new and effective antifungal treatment largely because of the lack of fungal-specific drug targets [15]. In a resource limited country like South Africa, the drugs most commonly used to treat invasive fungal infections are ABD and fluconazole. ABD has a broad spectrum of activity but is associated with side effects in 50-90% of patients, particularly nephrotoxicity or infusion related events [1, 20]. Fluconazole is well tolerated, with few drug interactions, but is inactive against molds. This agent is, however, often used as prophylaxis in hematology units to prevent

invasive yeast infections [1, 20]. The newer azoles and echinocandins have a wider spectrum of activity, which includes selected molds.

The treatment strategies in our study group were heterogeneous with 73.1% (19/26) of patients treated with antifungal therapy alone, 19.2% (5/26) with surgery alone and 7.7% (2/26) with a combined medical and surgical approach. Many patients received no treatment.

In the group of patients that were treated with antifungal agents and/or surgery 23% (6/26) demised during hospital stay. All of these patients had serious risk factors including aplastic anemia, hematological malignancy and primary immunodeficiency. Mucoraceous molds and *Aspergillus* species were the most common isolates in this group. All patients demised despite appropriate antifungal therapy with amphotericin B.

Of the patients who received antifungal therapy the majority received combination therapy. ABD was the most common antifungal agent used in combination therapy. Of note is that the majority of these patients received fluconazole as the second antifungal agent. The various combinations of antifungal drugs chosen varied widely between the patients, therefore, it is not possible to draw any further conclusions from the different combinations chosen. These findings reaffirm the challenges that clinicians face, not only in confirming the diagnosis of invasive fungal infections, but also in choosing the most appropriate antifungal therapy. Consultation with an infectious diseases specialist or microbiologist should therefore be considered for all patients with suspected invasive mold infections. Studies have reported improvement in antifungal therapy use as well as appropriate testing and follow up in those patients for which an infectious diseases specialist consultation was requested. These studies mainly included patients with candidemia, however, one would expect the same results in the context of invasive mold infections [21, 22].

Five patients were treated with surgery only. Four of these patients were diagnosed with nasal polyps and chronic allergic fungal rhinosinusitis. They all underwent surgical removal of the polyp. The use of antifungal agents in these patients remains controversial. The currently available data as well as consensus guidelines do not support the use of antifungal agents in patients with chronic allergic rhinosinusitis [23]. The fifth patient was diagnosed with a pulmonary apergilloma and underwent a fenestration procedure as suggested by the Infectious diseases Society of America's guidelines for the treatment of aspergillomas [24]. All 5 patients had favorable outcomes and were still being followed up at their respective clinics at the time the study was written up in 2018. Our study, therefore, reflects what is known about the management and outcome of these patients.

There were only 2 patients that were treated with a combination of antifungal therapy and a surgical intervention. The first patient had a hematological malignancy and developed a *Bipolaris* species fungal sinusitis. She received ABD as well as repeated surgical debridement and had a favorable outcome. Another patient had *Cladosporium* species cultured from 5 different tissue samples collected intra-operatively during a wash-out procedure for a prosthetic joint infection. She was treated with fluconazole as well as multiple courses of broad-spectrum antibiotics and clinically improved. She still followed up 3 years later. It is interesting to note that this patient improved following aggressive debridement despite

the lack of appropriate antifungal therapy. These cases highlight the important role for aggressive surgical intervention to ensure adequate source control.

In this study almost half of the patients received no treatment. Of these, 22.7% (5/22) had serious underlying risk factors and 27.3% (6/22) died, possibly before treatment could be initiated. One patient had a pulmonary aspergilloma and was managed conservatively with no change in her condition. In the remaining patients the isolated mold was considered to represent contamination. These included 8 nephrology patients receiving renal replacement therapy via peritoneal dialysis (PD) in whom a mold was cultured from PD fluid. Four of these patients had <100 polymorphonuclear cells indicating that the patients most likely did not have PD peritonitis. The remaining 4 had >100 polymorphonuclear cells, but cultured bacterial pathogens in addition to the mold, on either the same or a separate sample. In these 8 patients, the mold was also considered to be a contaminant. This reaffirms the view that the mere isolation of a fungal agent does not equate with disease and should always be correlated with clinical findings to determine the significance of the isolates [25, 26]. It is noteworthy that 4 of these samples were collected close to each other during February 2015, 2 on the same day in October 2015 and 2 during the same week in August 2016. This may indicate that infection control practices during sample collection may have been compromised. Contamination of clinical samples can occur at the time of specimen collection or during processing in the laboratory. Molds are environmental organisms and common contaminants in microbiology, which in most cases can be attributed to avoidable procedural errors. Proper specimen collection and correct laboratory practices is therefore of the utmost importance. One of the biggest issues confronting the cultivation of a mold is contamination by other molds [25, 26].

The overall in-hospital mortality was 25% in our study. A large multicenter study carried out in Asia published an overall mortality of 32.9% in 2018 [16]. There is a lack of data with regards to overall mortality in invasive mold infections inclusive of patients with all risk factors. The majority of available data reports on the mortality in specific groups of patients with specific risk factors. The mortality was slightly higher in the untreated group compared to the treated group. This difference did not reach statistical significance (p-value 0.31).

We found a significantly higher mortality rate in patients with underlying hematological conditions compared to the HIV positive group [14.3% (2/14) vs 41.7% (5/12)] which reflects what has been reported in the literature [19]. In the HIV positive group, the 2 patients who demised had very low CD4 counts of 30 and 50 cells/µl respectively, 9 had a favorable outcome and 3 were lost to follow up. These 3 patients had CD4 counts of 1, 13, 198 cells/µl respectively, were all started on antifungal therapy and referred back to their primary facilities. None of them attended their follow up appointments. It is possible that these patients demised. If this assumption was true, it would increase the mortality to 35.71% in the HIV positive group. It is worthy to note that, not only do these patients have very different underlying risk factors, but the type of molds identified was also very different and this may contribute to mortality.

Limitations of this study is the small sample size. The study was done retrospectively and most of the molds were reported to genus level only. Specific durations of therapy and reasons for chosen regimens could not be elucidated from the patient files as record keeping was poor in the majority of patients.

Conclusion

Fungal infections cause a high burden of disease in South Africa, driven largely by HIV, TB and poverty [27].

The diagnosis of invasive mold infections remains a challenge. In the current study, molds were found to cause serious infections, especially in at risk patients. However, molds are also common environmental organisms and therefore a common cause of contamination of clinical specimens, thus highlighting the need for clinical correlation in the interpretation of these results. Despite treatment with appropriate antifungal agents, the associated mortality rate was still high.

We therefore recommend that a clinical consultation with an infectious diseases specialist and microbiologist should be considered for all patients with suspected invasive mold infections.

We believe this is an important study that contributes to the growing knowledge on the distribution, patient characteristics and outcomes of invasive mold infections, particularly in patients in the Free State, and lays the foundation for further research in the field of invasive mold infections.

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A case series: The distribution, patient characteristics, therapy and patient outcome in culture positive invasive mold infections in HIV positive patients in a tertiary hospital in the Free State province, South Africa

Introduction

Fungi, including molds, are increasingly recognized as important pathogens in critically ill and immune compromised patients [1, 2]. Not only have these organisms assumed a greater role in human disease over the last 2 decades, but invasive fungal infections and in particular mold infections, are associated with significant morbidity and mortality [3, 4].

There is limited local data, regarding invasive mold infections especially in HIV positive patients, most likely due to diagnostic challenges.

We identified HIV as the most common risk factor (median CD4 88.5 cells/μl) a finding that has not been previously well documented in the literature. It should be noted that the background prevalence of HIV in the Free State is estimated at 5.1% as released in the midyear population estimates for 2018 by STATS SA (Statistics South Africa) [5].

Methods

With a retrospective review of laboratory and patient data, our study aimed to better quantify the distribution, patient characteristics, risk factors, therapy and treatment outcome in culture positive invasive mold infections at Universitas Academic Hospital (UAH) in the Free State province, South Africa between 1 July 2014 and 30 June 2017.

Case presentation

Patient 1
A 52-year-old male that presented with a history of crusted indurated plaques present over the forearms and face. He was newly diagnosed with HIV with a CD4 count of 13 cells/μl. *Sporothrix schenkii* was cultured on tissue samples and histology was positive for fungal elements. The patient received 14 days of intravenous ABD (amphotericin B deoxycholate) followed by oral itraconazole. He was down referred to his local primary care facility for palliative care. He never attended his follow up appointment.

Patient 2
A 52-year-old male presented with a nine month history of painful ulcerated large plaques on the face, limbs, trunk and back. He was HIV positive with a CD4 count of 65 cells/μl on antiretroviral therapy. *Sporothrix schenkii* was cultured on tissue samples and histological evaluation was suggestive of a deep fungal infection. He received 14 days of intravenous ABD followed by oral itraconazole. This patient responded well to therapy clinically as well as having documented negative follow up cultures.

Patient 3

A 42-year-old male presented with a thick confluent facial inflammatory plaque. He was newly diagnosed with HIV with a CD4 count of 21 cells/μl. *Sporothrix schenkii* was cultured on tissue samples and histological evaluation was suggestive of a deep fungal infection. He received 14 days of intravenous ABD followed by oral itraconazole. This patient responded well to therapy clinically.

Patient 4

A 44-year-old female patient presented with vulvar skin lesions. She was HIV positive on antiretroviral agents with a CD4 of 568 cells/μl. He was also known with metatstatic cervix carcinoma. A tissue biopsy was submitted for culture and histology. A *Bipolaris* species was cultured and histology was negative for fungal elements. In this patient the mold isolated was considered to be a contaminant and she did well despite not receiving any antifungal therapy.

Patient 5

A 28-year-old female patient presented with clinical features of sinusitis. She was a known HIV patient on antiretroviral therapy with a CD4 count of 484 cells/μl. She was also known with acute lymphocytic leukemia and was neutropenic on chemotherapy at the time of presentation. Middle turbinate tissue was submitted for culture and histology. A *Bipolaris* species was cultured and histology was suggestive of an invasive fungal infection. This patient received intravenous ABD as well as surgical debridement procedures (twice). She responded very well to the combined medical and surgical approach.

Patient 6

A 37-year-old male patient presented with a chronic fungal rhinosinusitis with maxillary sinus polyps. He was a known HIV positive patient on antiretroviral agents with a CD4 count of 328 cells/μl. The patient received endoscopic surgery to remove the polyps. Tissue was submitted for culture and histology. A *Bipolaris* species was cultured and histology was positive with numerous branching septate hyphae seen on fungal stains. The patient was treated with nasal saline and topical corticosteroids without receiving any antifungal therapy and had a favorable outcome. The use of antifungal agents in these patients' remains controversial. The currently available data as well as consensus guidelines do not support the use of antifungal agents in patients with chronic allergic rhinosinusitis.

Patient 7

A 36-year-old female patient presented with multiple firm nodules on the elbows, knees and distal lower legs and feet present for 6 months. She was HIV positive on antiretroviral therapy with a CD4 count of 142 cells/μl. Tissue biopsies were submitted for culture and histology. An *Aspergillus* species was cultured; however, histology was negative for fungal elements but suggestive of leprosy. This patient received therapy for leprosy as well as oral itraconazole. She responded well. The significance of the isolated mold in this case is questionable.

Patient 8

A 37-year-old male patient presented with lower limb skin lesions and one large, chronic, non-healing necrotic ulcer exposing his left foot tendons. He was HIV positive on antiretroviral therapy with a CD4 count of 291. Tissue specimens were submitted for culture and histology. An *Aspergillus* species was

isolated. Histology of the skin lesions was suggestive of syphilis. The patient received a surgical above-knee-amputation and was treated for syphilis. He had a favorable outcome. It is uncertain whether the ulcer was due to the mold isolated or if it was merely a contaminant.

Patient 9

A 41-year-old male presented with a history of an acute onset cough, fever and dyspnea. He deteriorated rapidly and required intubation and ICU care. Imaging was suggestive of a fungal infection of the lung. The patient was newly diagnosed HIV positive with a CD4 count of 30 cells/μl. An endotracheal aspirate sample was sent for culture and an *Aspergillus* species was isolated. The patient was initiated on intravenous ABD combined with voriconazole but despite appropriate therapy, demised 3 days after diagnosis.

Patient 10

A 33-year-old female patient presented with diffuse cutaneous lesions. She was newly diagnosed HIV positive with a CD4 count of 1 cell/μl. She was also co-infected with Hepatitis B virus. Tissue samples were submitted for culture and histology. *Histoplasma capsulatum* was isolated and histology was also suggestive of this infection. She received 14 days of intravenous ABD followed by 2 years of oral itraconazole. She had a favorable outcome.

Patient 11

A 30-year-old male patient He presented with multiple papules, some with a necrotic centers. Other papules were confluent and flat on the face and some were asymptomatic. The face, trunk, arms and legs were all affected. He was newly diagnosed HIV positive with a CD4 count of 1 cell/μl. An *Emmonsia* species was isolated and fungal stains were positive on histology. He received 14 days of intravenous ABD followed by oral itraconazole. The patient was down referred to his local primary care facility to continue his treatment. He never attended his follow up appointment.

Patient 12

A 10-year-old male patient presented with subdural empyema and cerebral abscesses complicating a *Streptococcus pneumoniae* meningitis. He was HIV positive with a CD4 count of 198 cells/μl. A *Penicillium* species was cultured from the subdural empyema fluid collected during a surgical washout procedure. The patient was transferred to his local primary care facility before the fungal results was available. He never attended his follow up appointment. It is uncertain what the outcome of this patient or the significance of the isolated mold was.

Patient 13

A 45-year-old female patient with Burkitt's lymphoma presented with diffuse skin lesions. She was newly diagnosed HIV positive with a CD4 count of 112 cells/μl. Tissue samples were submitted for culture only and histological evaluation was not requested. A *Cladosporium* species was isolated. In this patient the mold was considered to be a contaminant. She did not receive any antifungal therapy and had a favorable outcome.

Patient 14

A 32-year-old male patient presented with clinical features of rhino-orbital-cerebral mucormycosis. He

was newly diagnosed with HIV with a CD4 count of 50 cells/μl. Palate, cheek and nasal tissue was submitted for culture and histology. *Saksenaea oblongispora* was isolated and confirmed by molecular testing. Histology was positive for an angio-invasive fungal infection. He demised 3 days after the biopsies were submitted for evaluation. This patient was never started on any antifungal therapy and also did not receive any surgical intervention.

Table 1: Summary of findings

Mold species isolated	**Number (%)**
Sporothrix schenkii	3/14 (21.4)
Bipolaris species	3/14 (21.4)
Aspergillus species	3/14 (21.4)
Histoplasma capsulatum	1/14 (7.1)
Emmonsia species	1/14 (7.1)
Penicillium species	1/14 (7.1)
Cladosporium species	1/14 (7.1)
Saksenaea oblongispora	1/14 (7.1)
CD 4 count range	**Median**
1-568 cells/μl	88.5 cells/μl
Sex	**Number (%)**
Male	9/14 (64.3)
Female	5/14 (35.7)
Treatment	**Number (%)**
Antifungal agents	7/14 (50)
Surgery	2/14 (14.3)
Antifungal agents and surgery	1/14 (7.1)
None	4/14 (28.6)
Outcome	**Number (%)**
Favorable	9/14
Demised	2/14
Lost to follow up	3/14

Discussion

The mold species isolated from the 14 HIV positive patients were *Sporothrix schenkii*, *Bipolaris* species, *Aspergillus* species all at 21.4% (3/14), *Histoplasma capsulatum*, *Emmonsia* species, *Penicillium* species, *Cladosporium* species all at 7.1% (1/14). Interestingly a tissue sample from 1 patient with rhino-orbital-cerebral mucormycosis isolated a *Saksenaea oblongispora*, confirmed by PCR.

In the majority of patients 64.3% (9/14), the isolated mold was considered a significant pathogen. In these patients both culture and histology were positive. However, molds are also common environmental organisms and therefore a common cause of contamination of clinical specimens [6, 7].

An interesting and clinically very important finding is that 3 of these patients with very low CD4 cell counts of 65, 21, and 1 cells/μl respectively, responded very well to appropriate antifungal therapy and had a favorable outcome. Therefore, in this cohort, advanced disease did not equate to mortality. The importance of source control is also highlighted by the fact that all patients in this cohort that received a surgical intervention had a good outcome.

In this cohort of patients, 14.3% (2/14) died, 64.3% (9/14) had a favorable outcome and 21.4% of patients were lost to follow up. These 3 patients were all started on antifungal therapy and then referred back to their primary facilities. None of them attended their follow up appointments. It is possible that these patients could have demised. If this assumption is true, it would increase the mortality to 35.71% (5/14) in the HIV positive group.

Sporothrix schenkii affects all ages and genders but is usually associated with patients working in the agricultural sector (due to the main route of infection, traumatic inoculation, in these patients) and as expected, often males. The clinical presentation depends on the virulence of the organism, the size of inoculum and well as the patient's immune system. HIV-1 is associated with sporotrichosis with disseminated disease seen in the advanced stages if this infection [8, 9].

Bipolaris species forms part of the dematiaceous fungi, a heterogeneous group of fungi with dark colonies and pigmented fungal elements. Besides known to cause hypersensitivity reactions in susceptible individuals that sometimes lead to acute exacerbation of asthma, they are also important opportunistic pathogens in immunocompromised patients [10]. No clear correlation between *Bipolaris* species and HIV is documented in literature.

Chronic pulmonary aspergillosis is now emerging as a complication due to lung damage caused by HIV related pulmonary tuberculosis. Previously, pulmonary aspergillosis was seen mainly secondary to AZT-related neutropenia, however, cases may occur in patients with advanced immunodeficiency due to HIV-1 infection [11].

Disseminated histoplasmosis is a known AIDS-defining disease and roughly 25% of patients with stage 4 HIV-1 infection presents with this infection in endemic areas [11].

Penicilliosis is a well-known and common AIDS-defining infection in Southeast Asia, where this infection is endemic [11]. However, there is a lack of local data for infections caused by this mold.

Research done in South Africa, has recently shown an increase in patients presenting with an unmasking IRIS with *Emergomyces africanum* (formerly known as *Emmonsia* species) [12]. AIDS-related systemic mycoses is endemic to the western cape in south Africa and the most common species presenting with skin lesions identified in a study published in 2017 is *Emergomyces africanum*, *Histoplasma capsulatum* and *Sporothrix schenckii* [13].

A systematic review found that mucormycosis is a life-threatening infection in HIV infected individuals. This infection carries a very high mortality and is seen especially in patients also infected with other HIV-associated opportunistic infections and in those with very low CD4 cell counts. Predominant associated features were intravenous drug use, neutropenia and corticosteroid use [14]. *Sakseneae oblongispora* has recently been recognized as an emerging Zygomycete. This mucoraceous mold has been described to cause severe skin infections after traumatic implantation or surgery mostly in previously healthy patients and occasionally in patients with hematological conditions [15]. To our knowledge, this is the first case of invasive *Saksanaea oblongispora* infection described in in the setting of HIV.

Conclusion

The diagnosis of invasive mold infections remains a challenge. We therefore recommend that a high index of clinical suspicion is required and clinical consultation with an infectious diseases specialist and microbiologist should be considered for all patients with suspected invasive mold infections.

Important conclusions drawn from this cohort is that HIV infection is a common risk factor for invasive mold infections in South Africa. Advanced disease did not equate to mortality and the majority of patients responded well to appropriate therapy. The importance of source control was also demonstrated.

We believe it is an important study that contributes to the growing knowledge on the distribution, patient characteristics and outcomes of invasive mold infections, particularly in patients in the Free State, and lays the foundation for further research in the field of invasive mold infections.

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A case series: The distribution, patient characteristics, therapy and patient outcome in culture positive invasive mold infections in patients with hematological conditions in a tertiary hospital in the Free State province, South Africa

Introduction

Fungi, including molds, are increasingly recognized as important pathogens in critically ill and immune compromised patients [1, 2]. Not only have these organisms assumed a greater role in human disease over the last 2 decades, but invasive fungal infections and in particular mold infections, are associated with significant morbidity and mortality [3, 4].

There is limited local data, regarding invasive mold infections (IFIs), most likely due to diagnostic challenges.

We identified hematological conditions to be the second most common risk factor in patients with culture positive mold infections only second to HIV but the mortality in these patients were significantly higher compared to the patients with HIV (41.7% vs 14.3%).

Methods

With a retrospective review of laboratory and patient data, our study aimed to better quantify the distribution, patient characteristics, risk factors, therapy and treatment outcome in culture positive invasive mold infections at Universitas Academic Hospital (UAH) in the Free State province, South Africa between 1 July 2014 and 30 June 2017. This case series describes the cohort of patients affected by underlying hematological conditions.

Case presentation

Patient 1

A 19-year-old female known with aplastic anemia presented with nasal congestion and on examination she had necrotic nasal tissue. She was also neutropenic at the time of culture collection. Tissue samples were submitted for culture and an *Aspergillus* species was cultured. The patient was started empirically on intravenous ABD (amphotericin B deoxycholate) but demised on day 3 of therapy. She did not receive any surgical intervention.

Patient 2

A 58-year-old female known with AML (acute myeloid leukemia) presented with skin lesions. Tissue specimens were sent for culture and an *Aspergillus* species was isolated confirmed to be *Aspergillus flavus* by molecular methods. The sample was also submitted for bacteriological culture and *Pseudomonas aeruginosa* was isolated. The patient was treated with intravenous ABD followed by oral voriconazole. She also received antipseudomonal cover with appropriate antimicrobial agents, but she did not receive any surgical intervention. Unfortunately, the specimen was not submitted for histology. Histological examination could have increased the chances to identify the true pathogen/s. She, however, still demised, despite appropriate mold and bacterial cover.

Patient 3

A 22-year-old male patient known with AML presented with an ulcerative lesion on his lip. He was also neutropenic at the time of presentation. Tissue samples were submitted for culture and histology. An *Aspergillus* species was isolated, and histology was suggestive of an angio-invasive fungal infection. A surgical resection was done for this patient and he was treated with intravenous ABD followed with oral voriconazole. The patient responded well to the combined medical and surgical treatment approach and had documented negative follow up cultures.

Patient 4

A 53-year-old female known with ALL (acute lymphocytic leukemia) developed neutropenic sepsis during chemotherapy. She had multi-organ failure including respiratory failure. An *Aspergillus* species was isolated from her endotracheal aspirate sample. She also had a supporting 1, 3-beta-d-glucan of > 520 pg/ml. She was initiated on intravenous ABD but despite appropriate therapy demised 6 days after collection of the specimen.

Patient 5

A 23-year-old male patient known with aplastic anemia presented with clinical and radiological features of rhino-orbital-cerebral mucormycosis. Necrotic palate tissue was submitted for culture and histological evaluation. The patient was also neutropenic at the time of specimen collection. A mucoraceous mold confirmed as *Rhizomucor* species was isolated and fungal stains on histology revealed large angio-invasive fungal hyphae. The patient was empirically started on intravenous ABD. He did not receive any surgical intervention. The patient demised 2 days after collection of the specimens and initiation of antifungal therapy.

Patient 6

A 61-year old male known with myeloprolipherative disease presented with diffuse skin lesions. Tissue specimens were collected and sent for culture and histology. A mucoraceous mold was isolated however the histology was negative for fungal stains and suggestive of demodex mites. The isolated mold was considered a contaminant and the patient was not treated with any antifungal agents. The patient did not deteriorate acutely but passed away 1 month later. In this case the significance of the isolated mold is questionable.

Patient 7

A 35-year-old male known with AML presented with a chronic and non-healing ulcer on his left lower leg. A tissue specimen was sent for culture and histological examination. A *Fusarium* species was isolated. Histology was negative for fungal stains but positive for malignant cells compatible with acute myeloid leukemia. The clinical decision was made that the patient was for palliative care only and not for any escalation of therapy. He was therefore not treated with any antifungal agents. He, however, did not deteriorate and was subsequently discharged from hospital. He was admitted again 3 months later. Therefore, the significance of the isolated mold is doubtful.

Patient 8

A 28-year-old female patient presented with clinical features of sinusitis. She was a known HIV positive patient on antiretroviral therapy with a CD4 count of 484 cells/μl. She was also known with ALL and

was neutropenic on chemotherapy at the time of presentation. Middle turbinate tissue was submitted for culture and histology. A *Bipolaris* species was cultured and histology was suggestive of an invasive fungal infection. This patient received intravenous ABD as well as surgical debridement procedures (twice). She responded very well to the combined medical and surgical treatment approach.

Patient 9
A 25-year-old male patient known with AML presented with signs and symptoms of respiratory infection. He was also neutropenic on chemotherapy at the time of presentation. A brochoalveolar lavage was done for this patient and it was submitted for culture. A *Penicillium* species was isolated. The patient was treated with intravenous ABD followed by oral itraconazole and had a favorable response to therapy.

Patient 10
A 32-year-old male patient presented with generalized lymphadenopathy and skin lesions. A cervical lymph node biopsy and skin tissue was submitted for histology and fungal culture respectively. A *Chaetomium* species was isolated and confirmed with molecular methods on the skin tissue sample. Histology was negative for fungal stains and suggestive of angio-immunoblastic T-cell lymphoma on the lymph node biopsy. The isolated mold was considered to be a contaminant and the patient received no antifungal therapy. He had a favorable outcome despite receiving no mold cover.

Patient 11
A 45-year-old female patient with Burkitt's lymphoma presented with diffuse skin lesions. She was newly diagnosed HIV positive with a CD4 count of 112 cells/μl. Tissue samples were submitted for culture only, no histology was requested. A *Cladosporium* species was isolated. In this patient the mold was considered to be a contaminant. She did not receive any antifungal therapy and had a favorable outcome.

Patient 12
A 53-year old female known with ALL developed an episode of neutropenic fever during chemotherapy. Blood culture were taken and isolated a *Neurospora* species confirmed by molecular methods. She was initiated on intravenous ABD but demised only 1 day after initiation of therapy.

Discussion

IFIs represent an important cause of morbidity and mortality in patients with underlying hematological malignancies and still represents a major clinical problem as well as challenges in terms of the availability of and high costs related to the antifungal prophylaxis and treatment [5]. However, molds are also common environmental organisms and therefore a common cause of contamination of clinical specimens [6, 7].

When considering the high clinical diversity of these patients, the risk of IFIs may be very different for the different conditions and if such a risk is not appropriately evaluated, the possibility of overtreatment in some or under treatment in other patients is very likely [5].

It has been documented that IFIs are on the increase compared to invasive yeast infections that are decreasing [5]. There are many possible challenges contributing to this change in epidemiology in

patients affected by hematological conditions including selective pressure of antifungal prophylaxis, high numbers of high risk patients, a low threshold to start empiric therapy and limitations of currently available diagnostics [8].

Table 1. Summary of findings

Mold isolated	**Number (%)**
Aspergillus species	4/12 (33.3)
Mucoraceous molds	2/12 (16.7)
Fusarium species	1/12 (8.3)
Bipolaris species	1/12 (8.3)
Penicillium species	1/12 (8.3)
Chaetomium species	1/12 (8.3)
Cladosporium species	1/12 (8.3)
Neurospora species	1/12 (8.3)
Neutropenic at time of culture collection	**Number (%)**
Yes	8/12 (66.7)
No	4/12 (33.3)
Treatment	**Number (%)**
Antifungal agents	6/12 (50)
Antifungal agents PLUS surgery	2/12 (16.7)
None	4/12 (33.3)
Outcome	**Number (%)**
Favourable	7/12 (58.3)
Demised	5/12 (41.7)

The most common mold species isolated from the 12 patients with hematological conditions were *Aspergillus* species 33.3% (4/12), which is in keeping with that reported in the literature [9]. Studies have also shown that there is in increase in infections with Zygomycetes, *Fusarium* species and *Bipolaris* species [9]. This is in keeping with our findings with the mucoraceous molds being the second most common fungal isolate in this patient group. The majority of these patients with hematological conditions were neutropenic at 66.7% (8/12) at the time of culture collection and 16.2% (2/12) were known to be HIV positive.

Table 2. Distribution of hematological conditions

Hematological condition	Number of patients (Total = 12)
AML	4 (33.3%)
ALL	3 (25%)
Aplastic anemia	2 (16.7%)
Lymphoma	2 (16.7%)
Myeloprolipherative disease	1 (8.3%)

Table one describes the distribution of hematological conditions in our study with AML being the most common hematological condition in this study. This is in keeping with that reported by others. Patients with AML or patients treated with an allogeneic hematopoietic stem cell transplantation have an increased risk of IFIs. Moreover, some conditions predispose a high risk of IFIs, independently of the underlying disease, like neutropenia, relapse/refractory disease, previous history of IFIs, salvage therapy and therapy with high dose steroids [5].

The mortality in this group of patients was 41.7% (5/12). Almost all the patients that demised were neutropenic except for one patient with AML. In both patients with lymphoma the isolated mold was considered to be a contaminant and no conclusions could be drawn from them although it is known that lymphoma poses a lower risk of IFIs compared to AML [5].

All patients that required treatment were appropriately started on intravenous ABD, however, it is important to note that only 2 patients with skin and/or mucus membrane manifestations underwent a surgical procedure. Both of these patients had a good outcome with the combined medical surgical treatment approach. There were 3 patients that presented with skin and/or mucus membrane manifestations that did not undergo surgical debridement and despite appropriate antifungal therapy still demised. This emphasizes the important of source control in the management of IFIs.

Conclusion

IFIs are a major cause of morbidity and mortality in patients affected by hematological disorders, especially in the setting of neutropenia. However, molds are also common environmental organisms and therefore a common cause of contamination of clinical specimens, either at the time of specimen collection or in the laboratory itself [6, 7].

As with bacterial infections, local epidemiology should be considered before deciding on antifungal prophylaxis or empirical antifungal therapy. However, there is no local data available on IFIs in this setting.

Underlying haematological disorders and the type of IFI are important determinants of mortality [10]. Appropriate therapy is of major importance for the best possible outcome for these patients including appropriate surgical intervention when required.

We therefore recommend that a high index of clinical suspicion is required and clinical consultation with an infectious diseases specialist and microbiologist should be considered for all patients with suspected invasive mold infections.

We believe this is an important study that contributes to the growing knowledge on the distribution, patient characteristics and outcomes of invasive mold infections, particularly in patients in the Free State, and lays the foundation for further research in the field of invasive mold infections.

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Appendix a

UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
YUNIBESITHI YA FREISTATA

UFS-UV
HEALTH SCIENCES

FWA00012784

08 November 2017

DR BONITA VAN DER WESTHUIZEN
DEPT OF MEDICAL MICROBIOLOGY
FACULTY OF HEALTH SCIENCES
UFS

Dear Dr Bonita Van Der Westhuizen

HSREC 110/2017 (UFS-HSD2017/1122)
PRINCIPAL INVESTIGATOR: DR BONITA VAN DER WESTHUIZEN
PROJECT TITLE: THE DISTRIBUTION, PATIENT CHARACTERISTICS, THERAPY AND PATIENT OUTCOME IN CULTURE POSITIVE INVASIVE MOULD INFECTIONS IN A TERTIARY HOSPITAL IN THE FREE STATE PROVINCE, SOUTH AFRICA

APPROVED

1. You are hereby kindly informed that the Health Sciences Research Ethics Committee (HSREC) approved this protocol after all conditions were met. This decision will be ratified at the next meeting to be held on 05 December 2017.
2. The Committee must be informed of any serious adverse event and/or termination of the study.
3. Any amendment, extension or other modifications to the protocol must be submitted to the HSREC for approval.
4. A progress report should be submitted within one year of approval and annually for long term studies.
5. A final report should be submitted at the completion of the study.
6. Kindly use the **HSREC NR** as reference in correspondence to the HSREC Secretariat.
7. The HSREC functions in compliance with, but not limited to, the following documents and guidelines: The SA National Health Act. No. 61 of 2003; Ethics in Health Research: Principles, Structures and Processes (2015); SA GCP(2006); Declaration of Helsinki; The Belmont Report; The US Office of Human Research Protections 45 CFR 46 (for non-exempt research with human participants conducted or supported by the US Department of Health and Human Services-[HHS], 21 CFR 50, 21 CFR 56; CIOMS; ICH-GCP-EG Sections 1-4; The International Conference on Harmonization and Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH Tripartite), Guidelines of the SA Medicines Control Council as well as Laws and Regulations with regard to the Control of Medicines, Constitution of the HSREC of the Faculty of Health Sciences.

Yours faithfully

MS MGE MAAS
HEAD: HEALTH SCIENCES RESEARCH ETHICS COMMITTEE ADMINISTRATION

Health Sciences Research Ethics Committee
Office of the Dean: Health Sciences
T: +27 (0)51 401 7295/7794 | E: ethicsfhs@ufs.ac.za
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Appendix b

NATIONAL HEALTH LABORATORY SERVICE

Department of Medical Microbiology and Virology
Box 339 (G23), Faculty of Health Sciences, University of the
Free State, Bloemfontein, 9300
Tel: +27 (0)51 405 3162

3 April 2017

Dear Dr van der Westhuizen,

Re: Permission to perform study in the Department of Medical Microbiology and Virology

Approval is hereby granted to perform the study entitled "The distribution, patient characteristics, therapy and patient outcomes in culture positive invasive mould infections in a tertiary hospital in the Free State province, South Africa" in the Department of Medical Microbiology and Virology, National Health Laboratory Service/University of the Free State following approval by the NHLS Pathology Representative and the Health Sciences Research Ethics Committee.

Yours sincerely,

Dr Dominique Goedhals
Acting HOD/Pathologist: Department of Medical Microbiology and Virology

Appendix c

health

Department of Health

FREE STATE PROVINCE

09 October 2017

Dr B Van Der Westhuizen
Dept. of Medical Microbilogy
Faculty of Health Science
UFS

Dear Dr B Van Der Westhuizen

Subject: The distribution, patient characteristics, therapy and patient outcome in culture positive invasive mould infections in a tertiary hospital in the Free State province, South Africa.

- Please ensure that you read the whole document. Permission is hereby granted for the above – mentioned research on the following conditions:
- Serious Adverse events to be reported to the Free State department of health and/ or termination of the study
- Ascertain that your data collection exercise neither interferes with the day to day running of the Universitas Hospital nor the performance of duties by the respondents or health care workers.
- Confidentiality of information will be ensured and please do not obtain information regarding the identity of the participants.
- Research results and a complete report should be made available to the Free State Department of Health on completion of the study (a hard copy plus a soft copy).
- Progress report must be presented not later than one year after approval of the project to the Ethics Committee of the University of Free State and to Free State Department of Health.
- Any amendments, extension or other modifications to the protocol or investigators must be submitted to the Ethics Committee of the University of Free State and to Free State Department of Health.
- **Conditions stated in your Ethical Approval letter should be adhered to and a final copy of the Ethics Clearance Certificate should be submitted to** sebeclats@fshealth.gov.za **before you commence with the study**
- No financial liability will be placed on the Free State Department of Health
- Please discuss your study with the institution manager/CEOs on commencement for logistical arrangements
- Department of Health to be fully indemnified from any harm that participants and staff experiences in the study
- Researchers will be required to enter in to a formal agreement with the Free State department of health regulating and formalizing the research relationship (document will follow)
- You are encouraged to present your study findings/results at the Free State Provincial health research day
- Future research will only be granted permission if correct procedures are followed see http://nhrd.hst.org.za

Trust you find the above in order.

Kind Regards

Dr D Motau
HEAD: HEALTH
Date: 10/10/2017

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Appendix d

Protocol

Title

The distribution, patient characteristics, therapy and patient outcome in culture positive invasive mold infections in a tertiary hospital in the Free State province, South Africa.

Researchers

Main author: Dr B van der Westhuizen

Co-authors: Dr MS Abrahams, Dr S Potgieter

Co-supervisor: Prof Y Coovadia

Supervisor: Dr MS Abrahams

1. Introduction

Invasive fungal diseases are important causes of morbidity and mortality and, over the last two decades, have assumed a much greater importance, especially in immune compromised patients. Mold infections, in particular, have become more common [10, 12]. The patterns in antifungal resistance has also been changing [12, 13]. Fungi, including molds, are increasingly being recognized as major pathogens in critically ill and immunocompromised patients [1, 7].

Risk factors for developing invasive fungal infections include prolonged ICU stay, solid organ transplants, hematopoietic stem cell transplants, hematological malignancies, neutropenia, burn wounds, HIV infection, invasive medical devices and grafts. Additional risk factors in neonates include low birth weight and total parenteral nutrition. The use of antineoplastic and immunosuppressive agents, broad spectrum antibiotics and more aggressive surgery have also been identified as important contributing factors [1, 2, 4, 12].

Diagnosis of invasive fungal infections remains a challenge as the clinical manifestations are not specific. A histological diagnosis is often unfeasible. This lack of adequate diagnostic tests makes identification of the true disease burden due to fungal diseases difficult [5]. Alternatives to culture based methods, which has a low sensitivity, have been developed in an attempt to diagnose invasive fungal diseases earlier. The serial quantification of galactomannan has proven to be useful in the diagnosis of suspected *Aspergillus* infection. The use of 1, 3-Beta-D-Glucan has proven to be useful in diagnosing common invasive fungal infections in at risk patients. PCR-based methods should be considered as additional techniques for the early detection and confirmation of invasive mold infections, but their availability is currently limited to reference mycology laboratories [11].

In 2002, a consensus group of the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group (EORTC) and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (MSG) published standard definitions for invasive fungal infections for clinical and epidemiological research; these definitions were revised in 2008. These definitions include proven, probable and possible invasive fungal infections. Proven fungal infections are required to fulfill a mycological criterion from a sterile clinical sample (histology/culture). Probable invasive fungal infections are required to fulfill a mycological criterion from a non-sterile clinical sample, a host criterion and a clinical criterion. Possible invasive mold infections include only those cases with appropriate host factors, sufficient clinical evidence consistent with invasive fungal infections but without mycological support [3].

Candida species and *Cryptococcus* species have been identified as the most common causes of invasive yeast infections. These two fungi have been studied extensively, both internationally and locally. The most common moulds isolated in international studies are *Aspergillus* species, *Fusarium* species and *Rhizopus* species [1, 2, 5, 13]. In contrast, there is limited data on invasive mold infections with *Aspergillus* species, *Fusarium* species and *Rhizopus* species as the most common cause if those infections, locally.

There are large barriers for the development of new and effective antifungal treatment as fungi are eukaryotic pathogens and drug development is limited by a lack of fungal-specific drug targets [4]. In a resource limited country like South Africa, the most commonly used drugs to treat invasive fungal infections are Amphotericin B and Fluconazole. Amphotericin B has a broad spectrum of activity but side effects are common, occurring in 50-90% of patients, and are principally nephrotoxicity or infusion related events. Other side effects include fevers, chills, nausea, vomiting and hypotension. Nephrotoxicity is related to the use of other nephrotoxic agents and diuretics that is often required in the at risk population, and also to the dosage and duration of treatment [1, 8]. Liposomal Amphotericin B is less nephrotoxic but this formulation is expensive and not widely available [8]. Fluconazole is available in oral or intravenous formulations and is well tolerated, with few drug interactions. It is, however, inactive against molds. It is also often used as prophylaxis in hematology units to prevent invasive fungal infections [1, 8]. The newer azoles and echinocandins have a wider spectrum of activity against molds. Therefore, most patients in South Africa are treated with prolonged courses of Amphotericin B resulting in side effects [5].

Infections with drug-resistant organisms may lead to an especially poor outcome and a shift towards infections with azole and Amphotericin B resistant fungi has been observed [12, 13].

Despite general agreement that invasive fungal diseases are becoming more important, our understanding of these diseases remain incomplete, mostly due to the lack of surveillance data. Analysis of the US National Center for Health Statistics (NCHS) death records showed that fungal infections were the 7th most common cause of infectious disease-related mortality in 1992, and that fungal disease related fatalities had increased more than 3 fold since 1980. Additional analysis revealed that candidiasis and aspergillosis accounted for most of the deaths [2].

In this context, the Centre for Opportunistic, Tropical and Hospital infections at the National Institute of communicable Diseases, proposed in 2011 that the following fungal diseases be added to the list of Neglected Tropical Diseases (NTD's): Cryptococcal meningitis, *Pneumocystis jiroveci* pneumonia, Mycetoma, Histoplasmosis, Sporotrichosis and Blastomychosis. This would help to determine the prevalence, distribution and disease burden of these fungal diseases in sub-saharan Africa and provide information to prioritize strategies for control and prevention of these neglected fungal diseases [5]. In May 2016, the 69th World Health Assembly approved a resolution recognizing mycetoma as a neglected tropical disease [9].

Fungiscope is a Global Emerging Fungal Infection Registry that was established in 2003 to improve knowledge on epidemiology, clinical manifestations and treatment strategies for invasive fungal infections [6].

Currently, there are no recently published data on the distribution, treatment and patient outcomes of invasive mold infections in South Africa and the Free State in particular.

2. Aim

A retrospective data collection on the distribution, patient characteristics, therapy and patient outcomes in culture positive invasive mold infections at Universitas Hospital in the Free State province, South Africa.

3. Objectives

- To determine the spectrum of invasive mold infection pathogens in patients with culture positive invasive mold infections.
- To determine the baseline characteristics of patients with culture positive invasive mold infections.
- To assess the treatment and 12 week outcome in these patients.

4. Methods

4.1 Study design

Retrospective, observational descriptive study.

4.2 Target population

All culture positive samples from 1 July 2014 to 30 June 2017 will be included in this study. At least 60 specimens are expected to be included in this study.

4.3 Measurement

Culture positive molds from sterile sites will be included in this study. These isolates were cultured in the Universitas National Health Laboratory Service laboratory in Universitas hospital in the Free State,

South Africa. This is a SANAS accredited laboratory. All specimens have been processed according to the standard operating procedure in the laboratory. All specimens were planted onto 2 Sabouraud-Dextrose agar plates and incubated at 25 °C and 37 °C for 14 days. The plates were examined daily for fungal growth. When growth was observed, the macroscopic appearance of the isolate was described and a lacto-phenol-cotton-blue stain was performed for the microscopic examination of the mold.

The moulds from sterile sites were also sent to the National Institute of Communicable Diseases (NICD) for PCR testing. A pan-fungal PCR was performed for ITS (internal transcribed spacer) and large subunit of the ribosomal gene targets.
The following data will be included in the analysis:

- Mould identification.
- Patient age.
- Patient gender.
- Risk factors: Haematological malignancies, Bone-marrow transplant, solid organ transplant, neutropenia (<500 neutrophils/ml) > 10 days, ICU stay > 7 days, HIV status, CD4, immunosuppressive drugs, chemotherapy, corticosteroid therapy > 4 weeks or no risk factors.
- If the patient was treated with antifungal drugs.
- Which antifungal drug the patient was treated with.
- Patient outcome at 12 week follow up: Improvement, no change, deterioration.

Data will be collected from the electronic patient file system, Meditech, used at Universitas hospital. Patient clinic files will be used when the electronic information is not available. Patients for whom no clinical data is available will be excluded from the study. Data will be captured with the help of an Infectious Diseases Physician. Data will be collected on a Microsoft Excel spreadsheet approved by the department of biostatistics of the University of the Free State.

5. Analysis

Descriptive statistics, namely means and standard deviations or medians and percentiles, will be calculated for continuous data. Frequencies and percentages will be calculated for categorical data. The analysis will be done by the Department of Biostatistics.

6. Implementation of findings

Due to the paucity of data on invasive mold infections, this study will contribute to our knowledge on the distribution, patient characteristics and outcomes of invasive mold infections in patients in our setting and lay the foundation for further research in the field of invasive mold infections.

7. Time schedule

Literature review: December 2016 – January 2017

Protocol: February 2017 – April 2017

Protocol submitted to Department of biostatistics: April 2017 – May 2017

Protocol submitted for ethical approval: August 2017

DOH approval: September 2017 – October 2017

Data collection: November 2017 – December 2017

Write up and submit for M.Med marking: December 2017 – February 2018

Submit for publication: As marking is complete - March/April 2018

8. Budget

This is a retrospective data collection and a budget will only be required for stationary. The researcher will provide the budget.

- Paper and printing of data collection sheets – R200
- Miscellaneous stationary (pens, exam blocks) – R50

9. Ethical aspects

Consent will be obtained from the relevant heads of departments. The protocol will then be submitted for ethical approval and consent from the Department of Health.

10. References

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10. Appendices

Data capturing sheet

Letters of approval

Appendix e

Instructions

Mark the appropriate block with a X or write the answer on the space provided.

For Office Use

1 Date of data collection (dd/mm/yy)……

d d m m y y

2 Mould identification?

1	*Aspergillus* spp
2	*Fusarium* spp
3	*Rhizopus* spp
4	*Penicillium* spp
5	Other, specify……..

3 Patient gender

Male(1)	Female(2)

4 Patient age……..

5 Risk factors

1	HIV
2	Haematological malignancies
3	Bone-marrow transplant
4	Solid organ transplant
5	Neutropenia < 500 neutrophils/ml > 10 days
6	Prolonged ICU stay > 7 days
7	Immunosuppressive drugs
8	Chemotherapy
9	Corticosteroid therapy > 4 weeks
10	No risk factors

6 Was the patient treated?

1	Yes
2	No

7 What antifungal drug was used?

1	Amphotericin B
2	Fluconazole
3	Itraconazole
4	Voriconazole
5	Other, specify……

8 Patient outcome at 12 weeks

1	Improved
2	No change
3	Deteriorated
4	Patient was not followed up

Appendix f

Specimen type

1. Respiratory specimen
2. Tissue
3. Blood culture
4. Peritonial fluid
5. CSF

Identification (ID)

1. *Aspergillus* species
2. *Fusarium* species
3. Mucoraceous mold
4. *Bipolaris* species
5. *Penicillium* species
6. *Cladosporum* species
7. *Alternaria* species
8. *Histoplasma capsulatum*
9. *Sprothrix schenckii*
10. *Neurospora* species
11. *Saksenaea oblongispora*
12. *Chaetomium* species
13. *Phoma* species
14. *Emmonsia* species

Histology

1. Positive
2. Negative
3. Not applicable
4. Not sent

Gender

1. Male
2. Female

Risk factors (RF 1-4)

1. HIV
2. Hematological malignancy
3. Solid organ malignancy
4. Bone marrow transplant
5. Soft tissue transplant
6. Neutropenia
7. Prolonged ICU stay

8. Chemotherapy
9. Diabetes mellitus
10. Prolonged steroid therapy
11. Structural lung disease
12. Primary immunodeficiency
13. Aplastic anemia
14. Myeloprolipherative disease
15. Chronic kidney disease on peritoneal dialysis
16. Chronic sinusitis
17. Ventriculo-peritoneal shunt
18. None

Treated (Rx)

1. Yes
2. No

Drug used (Drug 1-3)

1. Amphotericin B
2. Fluconazole
3. Itraconazole
4. Voriconazole
5. Terbinafine

Surgery

1. Yes
2. No

Outcome

1. Improved
2. Unchanged
3. Deteriorated
4. Unknown

Appendix g

Author Guidelines

Manuscripts submitted to the SAJID must be in the form of *Research Articles, Brief Reports, Clinical Case Studies,*

Correspondence, Reviews, State-of-the-Art Articles, Commentaries and Opinion Papers, Editorials or *Supplement Articles.* The Journal welcomes the publication of *Guidelines, Conference Proceedings Newsletters or Press Releases*, and *Book Reviews.* Articles, Brief reports and Reviews are peer reviewed; other categories are reviewed by the Editors. Commentaries and Editorials are generally invited contributions, indicating the authors' identity, while manuscripts in the form of Reviews, and State-of-the-Art Articles may also be requested by the Editors.

All manuscripts must have conflict of interest and funding statements. When authors submit a manuscript, whether an article or a letter, they are responsible for disclosing all financial and personal relationships that might bias their work. To prevent ambiguity, authors must state explicitly whether potential conflicts do or do not exist. Authors should do so in the manuscript on a conflict-of-interest notification page that follows the title page.

Manuscripts describing research in human subjects or animals must indicate ethics clearance from appropriate research review committees. When reporting experiments on human subjects, authors should indicate whether the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. When reporting experiments on animals, authors should indicate whether the institutional and national guide for the care and use of laboratory animals was followed.

Articles describe original investigations at an acceptable degree of completion, constituting an advance in the field. Articles must not exceed 3500 words of text, without counting the abstract, references or legends, and illustrations and tables must be limited to the minimum necessary for clear and concise presentation. The abstract must either be structured, using *Background*, *Methods*, *Results*, and *Conclusions* as headings and comprising no more than 250 words, or unstructured with a 200 word limit. Articles are limited to a maximum of 7 insets (tables and figures combined) and 50 references.

Brief Reports present complete studies that are narrower in scope than those described in Articles or that present new developments. Manuscripts that are descriptive or primarily methodological in nature, or that describe in vitro chemotherapeutic studies should, in general, be submitted as Brief Reports. Brief Reports include an abstract (no more than 100 words) and are limited to a total of no more than 2000 words of text, a total of 2 inserts (tables or figures), and 15 references.

Correspondence (letters) must be submitted in reference to a previous publication in SAJID (within the previous 12 months), or relate to a topical matter in line with the interests of FIDSSA, PHASA or their affiliated societies. Please prepare the letter in manuscript format, including a title page. The letter must not exceed 750 words of text, 1 insert (table or figure) and 10 references.

Commentaries and Editorials are generally invited by the Editor and are overviews of articles in SAJID, or of other research in epidemiology or infectious diseases, or matters relating to public health and other issues of special interest to FIDSSA, PHASA or their associated societies. Unsolicited commentaries are also considered.

Reviews and *State-of-the-Art Articles* that are research oriented or fall within the fields of interests of FIDSSA, PHASA or any of their affiliated societies will be considered for publication by SAJID. Prospective authors of such manuscripts are advised to communicate with the Editor in advance to ensure that a specific contribution is deemed appropriate and timely. Manuscripts of Reviews and State-of-the-Art Articles will be peer-reviewed.

Reviewers

The Journal would encourage authors to supply the names of at least 2 potential reviewers for their manuscript, as well as to indicate any reviewers they would feel may have a potential conflict of interest with regard to their submission.

Supplements

Requirements for supplement manuscripts generally follow those for SAJID manuscripts, including conflict of interest and funding statements. Inquiries relating to suitability of topic, program organization, production and costs should be made to the Editor.

Evaluation of manuscripts

Review procedure. The Editor-in-Chief and Emeritus Editor screen all unsolicited manuscript submissions and some of these are rejected without further review. All other manuscripts are sent to a minimum of two outside experts for review. After receipt of the reviewers' reports, the Editor-in-Chief and the Emeritus Editor with administrative assistance of the Journal Secretary discuss the merits of the manuscripts and the Editor-in-Chief makes the final decision to accept, reject, or request revision of the manuscript. A request for revision does not guarantee ultimate acceptance of the revised manuscript

Related manuscripts. If there appears to be significant overlap between a manuscript submitted to SAJID and another submitted manuscript by the same authors to SAJID or another journal, the editors will take the matter up with the corresponding author, and based on the response, take appropriate action (ask for modification, or reject with detailed explanation). Further action may include informing the appropriate authority in the authors' resident institution and if overlapping is discovered after publication in SAJID, publishing an appropriate announcement to that effect in the journal.

DOCUMENT REQUIREMENTS

Checklist

The following are required for your manuscript to be processed:

- Covering letter
- Word count limits
- Conflict of interest statement
- Funding statement
- List of potential reviewers

Covering Letter

All manuscripts submitted to SAJID must be accompanied by a letter declaring that the manuscript has not been submitted or accepted for publication elsewhere. This letter must confirm and declare that all authors have seen and approved the content and have contributed significantly to the work. Authors should suggest potential unbiased reviewers who are qualified to review their manuscript. A covering letter must also accompany a revised submission and must address issues raised in the review process.

Manuscript Preparation

The SAJID complies with the Uniform Requirements for Manuscripts Submitted to Biomedical Journal Journals (*Ann Intern Med* 2000; 133:229-231 [editorial]; **http://www.icmje.org**, full text). Text, tables, references, and legends must be double- spaced. Italics should be used for genus and species names and for genes but not for in vivo, in vitro, in situ, et al., or other Latin-derived expressions. For layout of manuscript and appropriate style see a recent issue of SAJID.

Title page. On the title page, please supply a running head of not more than 40 characters and spaces, a title of not more than 160 characters and spaces, the names and affiliations of all the authors, and word counts of the abstract and text. Each author's first name, subsequent initials and surname must be used.

Footnote page. Footnotes must include:

- Statement that authors either have or have not a commercial or other association that might pose a conflict of interest (e.g. pharmaceutical stock ownership, consultancy, advisory board membership, relevant patents, or research funding)

- Statement naming sources of financial support (including grant numbers)

- Name, date (month and year), and location (city, and country if not South Africa) of a meeting at which all or part of the information has been presented (include an abstract number, if available)

- Name, address, telephone and fax numbers, and e-mail address of the person to whom correspondence should be addressed

- Current affiliations and addresses for authors whose affiliations have changed since completion of the study

Abstract. The abstract for an Article may be structured with the headings Background, Methods, Results, and

Conclusions (250-word limit) or unstructured (200-word limit). Abstracts of Brief Reports should be no more than 100 words. Whether structured or unstructured, the abstract must state the purpose of the research, the methods used, the results, and the conclusions. Do not cite references in the abstract. Include up to 10 key words, separate from the abstract. Please remember that the abstract is particularly useful for literature retrieval purposes.

Text. The text of Articles must be no longer than 3500 words, and that of Brief Reports no longer than 2000 words. The Methods section must include a statement that informed consent was obtained from patients or their parents or guardians, and human experimentation guidelines of the National Department of Health (http://www.doh.gov.za) or the South African Medical Research Council (MRC; http://www.sahealthinfo.org/ethics/index.htm) and /or those of the authors' institution(s) were followed in the conduct of clinical research or that animal experimentation guidelines (see MRC website above) were followed in animal studies.

References. Articles are generally limited to 50 references, Brief Reports to 15 references. Only works that have been published or accepted for publication can be included in the reference list. Unpublished observations by the authors (authors' unpublished data) personal communications (SP Stanley, personal communication), and manuscripts submitted for publication (J Odendaal, S Coovadia and J Radebe, submitted) should be mentioned parenthetically in the text Please number references in order of appearance; those cited only or first in tables or figures are numbered according to the order in which the table or figure is cited in the text. Example: If table 3 is cited in the text after reference 20, a new reference cited in table 3 will be reference 21.

References must follow the Uniform Requirements for Manuscripts Submitted to Biomedical Journals

(http://www.icmje.org, full text). Provide all authors' (or editors') names when there are fewer than 7; for 7 or more, list the first 3 and add "et al." Titles of journals not listed in *Index Medicus* should be spelt out in full. Reference to a doctoral thesis or Master's dissertation should include the author, title, institution, location, year and publication information, if published. For online resources, include a URL and date accessed. Accuracy of references is the responsibility of the authors.

Examples of the proper format are as follows:

Sonnenberg P, Glyn Thomas R, Glynn JR, Shearer S, Godfrey-Faussett, Murray J. Clinical and radiological features of pulmonary disease due to culture-positive Mycobacterium tuberculosis or non-tuberculous mycobacteria in South African gold miners. South Afr J Epidemiol Infect 2005; 20: 130-135

Marin M, Nguyen HQ, Langidrik JR, et al. Measles transmission and vaccine effectiveness during a large outbreak on a densely populated island: Implications for vaccination policy. Clin Infect Dis 2006; 42: 315-319

Strebel PM, Papania MJ, Halsey NA. Measles vaccine. In: Plotkin SA, Orenstein WA, eds. Vaccines. 4th ed. Philadelphia: WB Saunders, 2004: 389-440.

Mothibeli KM, McGee L, Smith AM, Klugman KP. Molecular epidemiology of pneumococcal serotype 3 isolates. [Abstract ID P56]. In: Programme and Abstract Book of the 1st Joint Congress of the Federation of Infectious Diseases Societies of Southern Africa (Sun City, North-West Province). Johannesburg: Presentations Graphics, 2005: 42.

World Health Organization. Initiative for vaccine research. Available at:

http://www.who.int/vaccine_research/diseases/measles/en/. Accessed 1 February 2005.

Acknowledgment(s). The page preceding the references may include a statement thanking those who assisted substantially with work relevant to the study.

Statistical analysis. The statistical analyses used should be identified both in the text and in all tables and figures where the results of statistical comparison are shown.

Units of measure. All Data should be expressed in metric units; use of SI units is encouraged. Use ºC for temperature.

Tables and figures. Articles are limited to a maximum of seven inserts (tables and figures combined), Brief Reports to a maximum of two inserts. Data should not be repeated in both a table and a figure. Abbreviations and acronyms used in tables and figures must be explained in the table footnotes and figure legends, even if already defined in the text.

Tables should be numbered in the order of mention in the text. Tables should be typed double-spaced throughout, with no vertical or internal rules. Footnotes and accompanying explanatory material should be kept to a minimum. Footnotes should be placed below the table and designated by superscript lowercase letters (listed in order of location when the table is read horizontally). Each column must have an appropriate heading describing the data in the column below, and units of measure must be clearly indicated. For further instructions on the preparation of tables in Word, consult the Special Instructions for Tables.

Figures should be also numbered in the order of mention in the text and should appear at the end of the manuscript and references. Your figures should be prepared in accordance with the Guidelines for Submission of Artwork. Letters, numbers, and symbols should be clear and of sufficient size to be legible when the figures are reduced. Photomicrographs should have internal scale markers. Figures reproduced from other publications must be accompanied by permission from the copyright holder. If the manuscript is accepted, the author will be required to send one complete set of glossy, hard-copy figures.

Figure legends should be double-spaced and appear on a separate page preceding the figures. Any abbreviations or symbols used but not defined in the figure itself must be defined in the legend.

Style. Authors are referred to the *American Medical Association Manual of style: A Guide for Authors and Editors* (9th ed., Williams& Wilkins, 1997) and the *Chicago Manual of Style* (15th ed., University of Chicago Press, 2003).

For commercially obtained products mentioned in the text, list the full names of manufacturers. Generic names of drugs and other chemical compounds should be used.

Nomenclature. SAJID recommends the latest widely accepted nomenclature, as set out in documents prepared by recognised international agencies e.g. the *International Journal of Systematic and Evolutionary Microbiology, Bergey's Manual of Determinative Bacteriology* (9th ed., revised, Williams& Wilkins, 1993), *Virus Taxonomy – The Classification and Nomenclature of Viruses: Sixth Report of the International Committee on Taxonomy of Viruses* (Springer-Verlag, 1995). The latter document also supplies standard abbreviations for virus species.

Clinical trials registration. All clinical trials must be registered in a registry that is electronically accessible to the public, free of charge. Registration should occur before patient enrolment and the registry's URL and the trial's registration number must be supplied at the end of the manuscript's abstract. For information on acceptable registries, consult the ICMJE Web site, http://www.icmje.org

. The National Library of Medicine's registry which is free and open to all investigators, generally meets with the requirements of journals for the publication of clinical trials.

MANUSCRIPT SUBMISSION

Procedure

Authors are advised to retain a copy of submitted manuscripts, including tables, figures and photomicrographs. The journal is not responsible for manuscripts lost or damaged.

All manuscripts must be submitted online at www.sajei.co.za. Register as an author and login. The covering letter must please be submitted as a supplementary file. For assistance to upload your manuscript or further instructions please contact Ms Robyn Marais at toc@sajei.co.za.

Appendix h

ARTICLE TYPES

Papers may be submitted in the following categories. The editors reserve the right to change the category for consistency with CID style.

Major Articles
Brief Reports
Review Articles
Viewpoints
Editorials
Invited Commentaries
Special Sections
Photo Quizzes
Correspondence Supplements

Major Articles

Report clinically relevant investigations or observations within CID s scope of interests.

Format guide:

- Word limit: 3000 words (excluding the abstract and references).
- Key points should be summarized on the title page in 40-words or less.
- References: 40 or less.
- Abstract: Up to 250 words, structured using the headings Background, Methods, Results and Conclusions.
- Tables/Figures: Data in the text should not be repeated extensively in tables or figures.

Brief Reports

Convey a focused message. Case reports are considered Brief Reports and have the same length requirements. Case reports should present unusual aspects of common problems or novel perspectives upon, or solutions to, clinically relevant issues.

Format guide:

- Word limit: 1500 words (excluding the abstract and references).
- References: 10-12.

- Abstract: Up to 50 words, unstructured format
- Tables/Figures: 1 table or figure.

Review Articles

Review topics should be related to clinical aspects of infectious diseases and should reflect trends and progress or a synthesis of data.

Format guide:

- Word limit: 3000 words (excluding the abstract and references).
- Key points should be summarized on the title page in 40-words or less.
- References: 40 or less.
- Abstract: Up to 150 words, unstructured.
- Tables/Figures: Data in the text should not be repeated extensively in tables or figures.

Viewpoints

We welcome viewpoints that present the opinions of the authors rather than new experimental data or literature reviews.

Format guide:

- Word limit: 3000 words (excluding the abstract and references).
- Key points should be summarized on the title page in 40-words or less.
- References: 40 or less.
- Abstract: Up to 150 words, unstructured.
- Tables/Figures: Data in the text should not be repeated extensively in tables or figures.

Editorials

Editorials relate to articles published in CID and are invited at the discretion of the Editor.

Please write a brief summary of your proposed editorial and email it to the editorial office.

Format guide:

- Word limit: 1200 words.
- Tables/Figures: A maximum of 1 figure or table.
- References: 10 or less.
- Ensure that there is a clear message in the conclusion.

Invited Commentaries

Invited Commentaries are timely and challenging aspects of Infectious Diseases, and are solicited by the Editor.

Format guide:

- Word limit: 1500 words.
- Abstract: Up to 50 words, unstructured.
- References: 40 or less.
- Tables/Figures: 1 table and/or figure.

Special Sections

Special Sections address areas of particular interest to CID and primarily contain articles solicited by the Special Section Editors from experts in each area.

Format guide:

- Word limit: 3000 words (excluding the abstract and references).
- Key points should be summarized on the title page in 40-words or less.
- References: 40 or less.
- Abstract: Up to 150 words, unstructured.
- Tables/Figures: Data in the text should not be repeated extensively in tables or figures.

Photo Quizzes

Photo Quizzes are question-and-answer challenges that illustrate an unusual illness and that invite a diagnosis from the reader. The question portion may state the history of the case and note the findings and the outcome, but it should not divulge the diagnosis.

Format guide:

- Question should be written in a single paragraph.

- Figures: 3 or less.
- Answer should give the diagnosis and outline or include the key teaching points.
- Separate figure legends for the question and answer must be included with the submission with arrows marking their key elements.

Correspondence

Letters to the Editor are intended primarily as a forum in which our readers may respond to a recently published article in CID. Replies will be published in the same issue as the letter, and are invited at the discretion of the Editor.

Format guide:

- Word limit: 500 words.
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- Begin with ‘Dear Editor’

Supplements

Supplements may be the proceedings of a symposium or the results of a study and are sponsored by universities, organizations, drug companies, and grants. Proposals related to suitability of topic, program organization, and production must be made in writing to the Editor.

Proposal format guide:

1. List of all topics to be covered
2. Estimated length of supplement
3. The complete contact information for a Guest Editor(s)
4. Potential authors
5. The name and contact information for the sponsoring organization

Appendix i

Lit review final

by Bonita Van Der Westhuizen

Submission date: 27-Mar-2019 03:12PM (UTC+0200)
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Chapter 1

Literature review

Invasive fungal infections (IFIs) are important causes of morbidity and mortality. IFIs have assumed a much greater importance in recent years. Mold infections, in particular, have become more common [1, 2]. This is mainly because of the increasing size of the population at risk. The most common causes of IFIs are *Candida albicans* and *Aspergillus* species. Different species are reported from different institutions. Other fungi that have increasingly been associated with infections in recent years include non-albicans *Candida* species, *Cryptococcus* and *Trichosporon,* molds such as *Fusarium* and the Zygomycetes. *Aspergillus fumigatus* is the most common cause of aspergillosis. Other *Aspergillus* species are less commonly encountered. Although an increase in aspergillosis is observed, there is a paucity of data on the proportions of infections caused by the different Aspergillus species [2].

Candida species and *Cryptococcus* [redacted] most common causes of invasive yeast [redacted]. These two fungi have been studied extensively, both internationally and locally. The most common molds isolated in international studies are *Aspergillus* species, *Fusarium* species and *Rhizopus* species [3, 4, 6, 7]. In contrast, there is limited local data, probably due to diagnostic challenges, on invasive mold infections, with these species. A recently published article based on results from Kwazulu-Natal, a province in South Africa, demonstrated that *Aspergillus* species were the most commonly isolated mold species among critically ill children [8]. More data have also become available about *Emergomyces africanum*, in South Africa, in the last few years. IFIs related to HIV infection are endemic to the Western Cape in South Africa and the most common species presenting with skin lesions identified in a study published in 2017 is *Emergomyces africanum*, *Histoplasma capsulatum* and *Sporothrix schenckii* [9]. Other research done in South Africa, has also shown an increase in patients presenting with an unmasking IRIS with *Emergomyces africanum* (formerly known as *Emmonsia* species) [10, 11].

Disseminated histoplasmosis is a known AIDS-defining disease that was classified as such in 1987 [12]. In 2013, Armstrong-James, D et al reported that roughly 25% of patients with stage 4 HIV-1 infection presents with this infection in endemic areas. *Histoplasma duboisi* have also became an important pathogen in patients with advanced HIV infection [13]. The biggest risk factor for histoplasmosis is the spread of HIV, although the use of immunosuppressive agents also contributes to this increase [12].

Chronic pulmonary aspergillosis is a common complication, increasingly seen, due to structural lung damage secondary to tuberculosis. Previously, pulmonary aspergillosis was mostly observed in patients who were neutropenic secondary to zidovudine therapy, but cases may also be seen in patients with advanced HIV infection [4]. It was recently estimated that the proportion of patients with chronic pulmonary aspergillosis in South Africa is at 175.8/100 000.This number is probably one of the highest in the world and it may partially be attributed to South Africa's high HIV infection and TB rate [14]. It is also a well-known fact that invasive aspergillosis is associated with hematological conditions [15].

Penicilliosis is also a common AIDS-defining infection in endemic Southeast Asia [4]. There is a paucity of local data on this particular mold.

Sporothrix schenckii infection is usually associated with individuals working in the agricultural sector and therefore mostly males. This is presumably due to the fact that traumatic inoculation is the most common route of infection. However, this mold may infect patients of all ages and genders. *Sporothrix schenkii* is associated with disseminated infection in patients with advanced HIV [16, 17].

A systematic review, including 61 articles, on mucormycosis in HIV infected patients published in 2016, have found that mucormycosis is a serious infection in HIV infected individuals. This infection carries a very high mortality and is seen especially in patients also infected with other HIV-associated opportunistic infections and in those with very low CD4 cell counts. Other important risk factors are diabetes mellitus, intravenous drug use, neutropenia and corticosteroid use [18].

Risk factors for developing IFIs include prolonged ICU stay, solid organ transplants, hematopoietic stem cell transplants, hematological malignancies, chemotherapy, immunosuppressive medication, neutropenia, burn wounds, HIV infection, invasive medical devices and grafts. Other important risk factors include low birth weight neonates and total parenteral nutrition. Broad spectrum antibiotics and more aggressive surgery have also been identified as important contributing factors [2, 4, 6, 13].

Although it is a well-known fact that invasive aspergillosis is associated with hematological conditions, studies have shown that there is in increase in infections with molds like the Zygomycetes, *Fusarium* species and *Bipolaris* species [15]. There are many different hematological conditions that can affect patients and the risk of developing systemic mycoses may be different for the different conditions. These risk factors need to be thoroughly evaluated as this may lead to inappropriate therapy in many patients resulting in over- or under treatment [19]. It has been documented that IFIs are on the increase compared to invasive yeast infections that are decreasing [19]. There are many possible challenges contributing to this change in epidemiology in patients affected by hematological conditions including selective pressure of antifungal prophylaxis, high numbers of high risk patients, low thresholds to start empiric therapy and limitations of currently available diagnostics [20]. Patients with an exceptionally high risk for developing IFIs are [redacted] myelocytic leukemia and allogeneic [redacted]. There are also additional conditions that predisposes these individuals to develop IFIs including neutropenia, relapse disease, refractory disease, previous history of IFIs and therapy with immunosuppressive agents [19].

The diagnosis of IFIs has proven to be very difficult, posing a diagnostic dilemma, as the clinical manifestations are not specific and the majority of available tests lack sensitivity. Due to these diagnostic challenges it is difficult to determine the true burden of disease due to these pathogens [7]. Culture has a suboptimal sensitivity, ranging from 8-62% for *Aspergillus* species and <25% for the mucoraceous molds, and may require incubation for several days to

weeks before growth is observed [21]. The sensitivity for culture from a sputum sample for acute pulmonary histoplasmosis is 10-15%, however, Wright-Giemsa stained peripheral blood smears are positive in up to 40% of these patients [12].A histological diagnosis is often not practical and the sensitivity of a histopathological examination for the identification of fungal elements is roughly 78% [22]. Alternatives to culture based methods, have been developed in an attempt to diagnose IFIs earlier. The use of 1, 3-Beta-D-Glucan has proven to be useful in diagnosing common invasive fungal infections in at risk patients, and the serial quantification of galactomannan has proven to be useful in the diagnosis of suspected *Aspergillus* infection. The pooled sensitivity of 1, 3-Beta-D-Glucan is around 76.8% and the specificity around 85.3%. 1,3-Beta-D-Glucan testing offers a much better sensitivity in comparison to culture, however, it is expensive, labour intensive and not widely available in most developing countries. 1, 3-Beta-D-Glucan testing is mainly helpful in the setting of its high negative predictive value and can be used to rule out invasive IFIs and withhold antifungal therapy but there are many causes (including antibiotics frequently used it this patient population) of false positive results [23]. The serum galactomannan test is very useful for neutropenic patients. This is largely due to its high negative predictive value (>98%).A positive serum galactomannan result in patients who are not neutropenic is of limited value due to its suboptimal sensitivity, however, it's detection in bronchoalveolar lavage specimens is very useful in non-neutropenic hosts [23]. PCR-based methods' major disadvantage is that it's availability is currently limited to reference mycology laboratories, however, it should be considered as a diagnostic tool when available [24]. A urine antigen test is available for the diagnosis of histoplasmosis. This test has recently been introduced at by the Mycology Reference Laboratory at NICD-NHLS. The test is still being validated and requires a urine specimen that has been refrigerated after collection [12]. With regards to aspergillosis, the most important laboratory test, IgA antibody, is not available in RSA [14].

Contamination of clinical samples with molds can occur any time from specimen collection to laboratory processing. Molds are one of the most common contaminants encountered in microbiology. Molds are environmental organisms and the spores of most molds are very light and easily transported by air. Therefore, just neglecting to clean a skin lesion properly before biopsy or just leaving the lid of a petri dish open for a few seconds, allows for the entry of contaminating organisms. The majority of contamination occurs through avoidable procedural errors. Proper specimen collection and correct laboratory practices is of utmost importance if an invasive mold infection is suspected. One of the biggest issues confronting the cultivation of a mold is contamination by other molds [25, 56].

Definitions for IFI's for clinical and epidemiological research were published in 2002; these definitions were revised in 2008. These definitions include proven, probable and possible IFIs. Proven fungal infections are required to fulfill a mycological criterion from a sterile clinical sample (histology/culture). Probable IFIs are required to fulfill a mycological criterion from a non-sterile clinical sample, a host criterion and a clinical criterion. Possible IFIs include only those cases with risk factors, clinical signs and symptoms consistent with IFIs but without mycological support [27].

The development of new and effective antifungal treatment is hampered by the fact that fungi are eukaryotic organisms and therefore drug development is limited by the lack of fungal-specific drug targets [13]. In a resource limited country like South Africa, the most commonly used drugs to treat IFIs are amphotericin B (ABD) and fluconazole. ABD has a broad spectrum of activity, but side effects are common, occurring in more than half of the patients. Side effects are mainly nephrotoxicity or infusion related events. Other side effects include hypokalemia, fevers, nausea, vomiting and hypotension. Nephrotoxicity is aggravated by the use of other drugs that affects renal function and diuretics, that are often required in the population at risk, as well as the dosage and the duration of treatment required [4, 28]. Liposomal ABD is less nephrotoxic, but this formulation is expensive and not widely available [28]. Fluconazole is well tolerated, with few drug interactions, but is inactive against molds. However, it is often used as prophylaxis in hematology units to prevent invasive yeast infections [1, 28]. The newer azoles and echinocandins have a wider spectrum of activity, which includes selected molds. Therefore, most patients in South Africa are treated with prolonged courses of ABD with its associated side effects [7].

The patterns in antifungal resistance has also been changing [2, 3]. Advances made in clinical medicine have led to changes in the incidence and drug resistance of fungal infections. This includes the practice where ABD is the main drug used for empiric therapy against IFIs in most centers and azoles are used more frequently for antifungal prophylaxis in at risk patients. A shift towards infections with azole and ABD resistant fungi has been reported, which may be associated with especially poor outcomes [2, 3].

Despite general agreement that invasive fungal diseases are becoming more important, our understanding of these diseases remain incomplete, mainly due to the lack of surveillance data. Analysis of death records in the USA showed that fungal infections were the 7th most common cause of death due to infections in 1992, and that mortality secondary to IFIs has increased significantly since 1980. Candidiasis and aspergillosis accounted for most of these deaths [6].

In this context, it was proposed in 2011, that the following fungal diseases be added to the list of Neglected Tropical Diseases (NTD's): Cryptococcal meningitis, *Pneumocystis jiroveci* pneumonia, Mycetoma, Histoplasmosis, Sporotrichosis and Blastomycosis. This would help to determine the epidemiology and burden of disease. This information will also allow the development of strategies to control and prevent of these neglected fungal diseases [7]. In May 2016 mycetoma was acknowledged as a neglected tropical disease [29].

Fungiscope is a Global Emerging Fungal Infection Registry that was established in 2003 to improve knowledge on epidemiology, clinical manifestations and treatment strategies for invasive fungal infections [30].

There is very limited published data on the distribution, treatment and patient outcomes of IFIs in South Africa, and the Free State in particular.

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Appendix j

Article final

by Bonita Van Der Westhuizen

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The distribution, patient characteristics, therapy and patient outcome in culture positive invasive mold infections in a tertiary hospital in the Free State province, South Africa.

Word count - 3471

Introduction

Fungi, including molds, are increasingly recognized as important pathogens in critically ill and immune compromised patients [1, 2]. Not only have these organisms assumed a greater role in human disease over the last two decades, but invasive fungal infections, and in particular mold infections, are associated with significant morbidity and mortality [3, 4].

Despite general agreement that invasive fungal diseases are becoming more important, our understanding of these diseases remain incomplete, mainly due to the lack of surveillance data. The most common isolated molds in international studies are *Aspergillus* species, *Fusarium* species and mucoraceous molds [1, 5, 6, 7]. A recently published article from Kwazulu-Natal, South Africa reported that *Aspergillus* species was the most commonly isolated mold species amongst critically ill children [8]. Recent studies done in South Africa reported an increase in HIV positive patients presenting with an unmasking IRIS with *Emergomyces africanum* (formerly known as *Emmonsia* species) [9, 10]. IFIs associated with HIV infection is endemic to the Western Cape province in South Africa with the most common species presenting with skin lesions identified in a study published in 2017 being *Emergomyces africanum*, *Histoplasma capsulatum* and *Sporothrix schenckii* [11]. Except for these publications, there is limited local data, regarding invasive mold infections, most likely due to diagnostic challenges.

With a retrospective review of laboratory and patient data, this study aimed to better quantify the distribution, patient characteristics, risk factors, therapy and treatment outcome in culture positive invasive mold infections at Universitas Academic Hospital (UAH) in the Free State province, South Africa.

Methods

Study design and setting

A retrospective, observational descriptive study was performed. Patients admitted to UAH between 1 July 2014 and 30 June 2017, in whom a mold was isolated from fungal culture, were included in the study. UAH is the only tertiary referral hospital providing specialist and sub-specialist level care for the Free State and Northern Cape provinces.

All culture positive molds from sterile sites (tissue specimens, blood cultures, peritoneal fluid and cerebrospinal fluid) were included in the final analysis. Although bronchoalveolar lavage fluid, endotracheal aspirates and sputum specimens are not considered sterile specimens, they were still included in the study if the same mold was cultured from a second specimen, and the patient had symptoms of an invasive fungal disease of the lungs together with supporting clinical and/or radiological signs.

We excluded all specimens that were negative for fungal growth, specimens that cultured a yeast, specimens that cultured a mold from a non-sterile site (except for selected respiratory specimens) and non-human specimens.

Sample Analysis

Isolates were cultured at the Universitas National Health Laboratory Service Microbiology Laboratory, a SANAS (South African National Accreditation System) accredited laboratory. All specimens had been processed according to the standard operating procedure in the laboratory [12]. Specimens were inoculated onto 2 Sabouraud-Dextrose agar plates and incubated at 25 °C and 37 °C respectively for 14 days. The plates were examined daily for fungal growth. When growth was observed, the macroscopic appearance of the isolates was described, and a lacto-phenol-cotton-blue stain was performed for the microscopic examination to determine the identity of the mold. The molds that proved difficult to identify in our laboratory, were sent to the mycology reference laboratory at the NICD (National Institute of Communicable Diseases) for broad-range fungal polymerase-chain-reaction.

Data Collection

Laboratory results were obtained from the Central Data Warehouse at the NICD. All specimens registered for mycology culture from UAH for the period 1 July 2014 to 30 June 2017 were reviewed. Patient information was collected from the electronic patient file system, Meditech, used at UAH.

Patient clinic and hospital files were used when the electronic information was not available. Patients for whom no clinical data was available were excluded from the study. Data was captured with the help of an Infectious Diseases Physician, on a Microsoft Excel spreadsheet approved by the department of biostatistics of the University of the Free State.

Data Analysis

Descriptive statistics namely means and standard deviations or medians and percentiles, were calculated for continuous data. Frequencies and percentages were calculated for categorical data. The analysis was performed by the Department of Biostatistics.

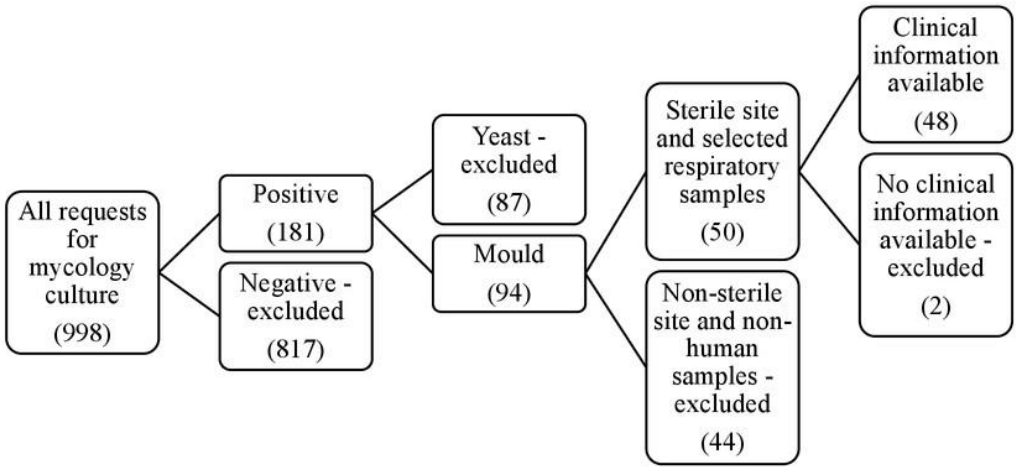
Ethical aspects

Approval to conduct the study was obtained from the NHLS business manager and acting Head of Department for Medical Microbiology as well as the Free State Department of Health, the Health Sciences Research Ethics Committee of the University of the Free State (HSREC 110/2017, UFS-HSD2017/1122). Confidentiality was ensured by allocating a number to each patient's record and by excluding all patients' personal information.

Results

From a total of 998 samples submitted for mycology culture, 950 were excluded based on the above described exclusion criteria as depicted in figure 1.

Figure 1. Patient selection



The remaining 48 samples were followed up and the results are depicted in Tables below.

Table 1. Summary of specimen type

Outcome	**Number of specimens**	**Percentage of specimens**
Specimen type	**Sample size 48**	**%**
Respiratory specimens	8	16.7
Tissue	29	60.4
Blood culture	1	2.1
Peritoneal fluid	8	16.7
Cerebrospinal fluid	2	4.2

Table 1 summarizes specimen distribution of the culture positive samples. Tissue was the most common sample type from which a mold was isolated, followed by respiratory samples and peritoneal dialysis fluid.

The presence of invasive fungal infection was confirmed upon histological examination in 37.9% (11/29) tissue samples from which a mold was cultured. In 20.7% (6/29) of the tissue specimens, histological evaluation was not requested.

It is noteworthy, that although the tissue samples that were negative for fungal growth were not included in this study for further analysis, roughly 70% of these were also submitted for histological examination. Upon histological examination 40% of these tissue samples were negative for fungal elements, approximately 10% were positive for fungal elements and the rest were suggestive of other pathology, namely malignancies, syphilis and drug reactions. Less frequently suggestive diagnoses included *Mycobacterium tuberculosis*, Mycobacteria other than *Mycobacterium tuberculosis*, *Echinococcus granulosis* and various other skin conditions.

Table 2. Baseline characteristics of the patients

Outcome	**Number of patients**	**Percentage of patients**
Sex	**Sample size 48**	**%**
Male	24	50
Female	24	50
Risk factor		
HIV	14	29.2
Hematological malignancy	9	18.8
Neutropenia	8	16.7
Chemotherapy	8	16.7
Chronic kidney disease on Peritoneal dialysis	8	16.7
Solid organ malignancy	3	6.3
Structural lung [redacted]	3	[redacted]
[redacted]	2	[redacted]
Aplastic anemia	2	4.2
Allergic rhinosinusitis with nasal polyps	2	4.2
Steroid therapy	1	2.1
Primary immunodeficiency	1	2.1

Myeloproliferative disease	1	2.1
Ventriculo-peritoneal shunt	1	2.1
None identified	5	10.4

Table 2 summarizes the baseline patient characteristics. Males and females had equal numbers of positive samples and the median age was 40.5 [redacted] 7-78 years). The most common risk factor was HIV with only 10.4% (5/48) of patients having no identifiable risk factors. The median CD4 count in the HIV positive patients was 88.5 cells/µl (range 1-568 cells/µl). There were no patients that received bone marrow or solid organ transplants and no patients fulfilled the criteria for prolonged ICU stay defined as > 7 days.

Table 3. Mold species isolated

Outcome	**Number of specimens**	**Percentage of specimens**
Species isolated	**Sample size 48**	**%**
Aspergillus species	19	39.6
Fusarium species	5	10.4
Bipolaris species	5	10.4
Mucoraceous molds	4	8.3
Cladosporium species	3	6.3
Sporothrix schenckii	3	6.3
Penicillium species	2	4.2
Alternaria species	2	4.2
Histoplasma capsulatum	1	2.1
Neurospora species	1	2.1
Chaetomium species	1	2.1
Phoma species	1	2.1
Emmonsia species	1	2.1

Table 3 describes the mold distribution of culture positive isolates. *Aspergillus* species was by far the most commonly isolated mold followed by *Fusarium* species, *Bipolaris* species and the mucoraceous molds.

Table 4. Summary of treatment results

Outcome	Number of patients	Percentage of patients
Treatment (antifungal agent/s and/or surgery)	**Sample size 48**	**%**
Yes	26	54.2
No	22	45.8
Treatment modality		
Antifungal therapy alone	19	39.6
Surgery alone	5	10.4
Surgery plus antifungal therapy	2	4.2
Antifungal agents		
ABD	17	35.4
Fluconazole	7	14.6
Itraconazole	7	14.6
Voriconazole	5	10.4
Terbinafine	1	2.1

Table 4 summarizes the treatment modalities chosen for each patient. The majority of patients were treated with antifungal therapy alone and of these 78.9% (15/19) received combination therapy, mostly with ABD (Amphotericin B deoxycholate) combined with an azole. Only 26.9% (7/26) of patients received surgical intervention, with 2 receiving additional antifungal therapy.

Table 5. Outcome

Outcome	Number of patients	Percentage of patients
Patient response	**Sample size 48**	**%**
Improved	32	66.7
Unchanged	1	2.1
Demised in hospital	12	25
Unknown	3	6.3

Table 5 describes the patient outcome in our study. Most of the patients had a favorable outcome but the in-hospital mortality was 25%. The mortality rate amongst the treated patients was 23.1% (6/26) and 27.3% (6/22) in the untreated group (p-value 0.31).

Discussion

Diagnosis of invasive fungal infections remains a challenge, as [redacted] manifestations are often non-specific. A lack of reliable diagnostic testing makes it difficult to estimate the true burden of fungal disease [6]. The reported sensitivity of histopathological methods for diagnosing invasive fungal infections is approximately 78%, as compared to 8-60% for culture [13, 14].

In our study 37.9% (11/29) had positive histology, but 20.7% (6/29) of tissue samples collected were not submitted for histological examination, thus probably underestimating the true sensitivity of histology. Amongst the culture negative samples, a large number of those submitted for histology identified another pathology and some identified an invasive mold infection. Although the sensitivity of histological identification of invasive mold infection in our study was low, it is clear that histology is helpful in diagnosing additional invasive fungal infections that may have been missed by culture as well as for diagnosing other infectious and non-infectious conditions. This underscores the need for closer interaction between the laboratory and clinicians regarding the submission of appropriate specimens, especially for difficult to diagnose infections.

In keeping with the findings of studies performed internationally, we also found that *Aspergillus* species followed by *Fusarium* species, *Bipolaris* species and the mucoraceous molds were the most common fungi isolated [1, 5, 6, 7]. Enoch *et al* reported that the most common mold isolated in the UK is *Aspergillus* species, although *Fusarium* species, *Scedosporium* species, *Penicillium* species and the Zygomycetes are increasingly seen [1]. Malani *et al* reported a similar picture in the USA [7].

Risk factors for developing [redacted] ICU stay, solid organ transplants, hematopoietic stem cell transplants, hematological malignancies, neutropenia, burn wounds, HIV infection, invasive medical devices and grafts. The use of antineoplastic and immunosuppressive agents, broad spectrum antibiotics and more

aggressive surgery have also been identified as important contributing factors [1, 4, 5, 15]. The risk factors identified in our study population generally reflect those reported by others, except for HIV that is the most common risk factor in our study. The risk factors identified in international studies are diverse and in most patients multiple, however, hematopoietic stem cell transplant recipients, patients with hematological malignancies and neutropenia are described as the most common risk factors. A large multicenter trial done in Asia identified prolonged corticosteroid use as the most common risk factor to develop IFI's in their setting [16].

We identified HIV as the most common risk factor (median CD4 88.5 cells/µl), a finding that has previously not been well documented in the literature. This may be due to the paucity of data from countries with a high burden of HIV infection. It should be noted that the background prevalence of HIV in the Free State is estimated at 5.1% as released in the midyear population estimates for 2018 by STATS SA (Statistics South Africa) [17]. The mold species isolated from the 14 HIV positive patients were *Sporothrix schenkii*, *Bipolaris* species, *Aspergillus* species all at 21.4% (3/14), *Histoplasma capsulatum*, *Emmonsia* species, *Penicillium* species, *Cladosporium* species all at 7.1% (1/14). Interestingly a tissue sample from 1 patient with rhino-orbital-cerebral mucormycosis isolated a *Saksenaea oblongispora*, confirmed by PCR. This organism has recently been recognized as an emerging Zygomycete [18]. To our knowledge, this is the first case of invasive *Saksanaea oblongispora* infection described in the setting of HIV.

The most common mold species isolated from the 12 patients with hematological conditions were *Aspergillus* species 33.3% (4/12), which is in keeping with that reported in the literature. Studies have also shown that there is in increase in infections with Zygomycetes, *Fusarium* species and *Bipolaris* species [19]. This is in keeping with our findings with the mucoraceous molds being the second most common fungal isolate in this patient group. The majority of these patients with hematological conditions were neutropenic at 66.7% (8/12) at the time of culture collection and 16.2% (2/12) were known to be HIV positive.

There are many barriers to the development of new and effective antifungal treatment largely because of the lack of fungal-specific drug targets [15]. In a resource limited country like South Africa, the drugs most commonly used to treat invasive fungal infections are ABD and fluconazole. ABD has a broad spectrum of activity but is associated with side effects in 50-90% of patients, particularly nephrotoxicity or infusion related events [1, 20]. Fluconazole is well tolerated, with few drug interactions, but is inactive against molds. This agent is, however, often used as prophylaxis in hematology units to prevent invasive yeast infections [1, 20]. The newer azoles and echinocandins have a wider spectrum of activity, which includes selected molds.

The treatment strategies in our study group were heterogeneous with 73.1% (19/26) of patients treated with antifungal therapy alone, 19.2% (5/26) with surgery alone and 7.7% (2/26) with a combined medical and surgical approach. Many patients received no treatment. In the group of patients that were treated with antifungal agents and/or surgery 23% (6/26) demised during hospital stay. All of these patients had serious risk factors including aplastic anemia, hematological malignancy and primary immunodeficiency. Mucoraceous molds and

Aspergillus species were the most common isolates in this group. All patients demised despite appropriate antifungal therapy with amphotericin B.

Of the patients who received antifungal therapy the majority received combination therapy. ABD was the most common antifungal agent used in combination therapy. Of note is that the majority of these patients received fluconazole as the second antifungal agent. The various combinations of antifungal drugs chosen varied widely between the patients, therefore, it is not possible to draw any further conclusions from the different combinations chosen. These findings reaffirm the challenges that clinicians face, not only in confirming the diagnosis of invasive fungal infections, but also in choosing the most appropriate antifungal therapy. Consultation with an infectious diseases specialist or microbiologist should therefore be considered for all patients with suspected invasive mold infections. Studies have reported improvement in antifungal therapy use as well as appropriate testing and follow up in those patients for which an infectious diseases specialist consultation was requested. These studies mainly included patients with candidemia, however, one would expect the same results in the context of invasive mold infections [21, 22].

Five patients were treated with surgery only. Four of these patients were diagnosed with nasal polyps and chronic allergic fungal rhinosinusitis. They all underwent surgical removal of the polyp. The use of antifungal agents in these patients remains controversial. The currently available data as well as consensus guidelines do not support the use of antifungal agents in patients with chronic allergic rhinosinusitis [23]. The fifth patient was diagnosed with a pulmonary apergilloma and underwent a fenestration procedure as suggested by the Infectious diseases Society of America's guidelines for the treatment of aspergillomas [24]. All 5 patients had favorable outcomes and were still being followed up at their respective clinics at the time the study was written up in 2018. Our study, therefore, reflects what is known about the management and outcome of these patients.

There were only 2 patients that were treated with a combination of antifungal therapy and a surgical intervention. The first patient had a hematological malignancy and developed a *Bipolaris* species fungal sinusitis. She received ABD as well as repeated surgical debridement and had a favorable outcome. Another patient had *Cladosporium* species cultured from 5 different tissue samples collected intra-operatively during a wash-out procedure for a prosthetic joint infection. She was treated with fluconazole as well as multiple courses of broad-spectrum antibiotics and clinically improved. She still followed up 3 years later. It is interesting to note that this patient improved following aggressive debridement despite the lack of appropriate antifungal therapy. These cases highlight the important role for aggressive surgical intervention to ensure adequate source control.

In this study almost half of the patients received no treatment. Of these, 22.7% (5/22) had serious underlying risk factors and 27.3% (6/22) died, possibly before treatment could be initiated. One patient had a pulmonary aspergilloma and was managed conservatively with no change in her condition. In the remaining patients the isolated mold was considered to represent contamination. These included 8 nephrology patients receiving renal replacement therapy via peritoneal dialysis (PD) in whom a mold was cultured from PD fluid. Four of these patients had <100 polymorphonuclear cells indicating that the patients most likely did

not have PD peritonitis. The remaining 4 had >100 polymorphonuclear cells, but cultured bacterial pathogens in addition to the mold, on either the same or a separate sample. In these 8 patients, the mold was also considered to be a contaminant. This reaffirms the view that the mere isolation of a fungal agent does not equate with disease and should always be correlated with clinical findings to determine the significance of the isolates [25, 26]. It is noteworthy that 4 of these samples were collected close to each other during February 2015, 2 on the same day in October 2015 and 2 during the same week in August 2016. This may indicate that infection control practices during sample collection may have been compromised. Contamination of clinical samples can occur at the time of specimen collection or during processing in the laboratory. Molds are environmental organisms and common contaminants in microbiology, which in most cases can be attributed to avoidable procedural errors. Proper specimen collection and correct laboratory practices is therefore of the utmost importance. One of the biggest issues confronting the cultivation of a mold is contamination by other molds [25, 26].

The overall in-hospital mortality was 25% in our study. A large multicenter study carried out in Asia published an overall mortality of 32.9% in 2018 [16]. There is a lack of data with regards to overall mortality in invasive mold infections inclusive of patients with all risk factors. The majority of available data reports on the mortality in specific groups of patients with specific risk factors. The mortality was slightly higher in the untreated group compared to the treated group. This difference did not reach statistical significance (p-value 0.31).

We found a significantly higher mortality rate in patients with underlying hematological conditions compared to the HIV positive group [14.3% (2/14) vs 41.7% (5/12)] which reflects what has been reported in the literature [19]. In the HIV positive group, the 2 patients who demised had very low CD4 counts of 30 and 50 cells/μl respectively, 9 had a favorable outcome and 3 were lost to follow up. These 3 patients had CD4 counts of 1, 13, 198 cells/μl respectively, were all started on antifungal therapy and referred back to their primary facilities. None of them attended their follow up appointments. It is possible that these patients demised. If this assumption was true, it would increase the mortality to 35.71% in the HIV positive group. It is worthy to note that, not only do these patients have very different underlying risk factors, but the type of molds identified was also very different and this may contribute to mortality.

Limitations of this study is [redacted] study was done retrospectively and most of the molds were reported to genus level only. Specific durations of therapy and reasons for chosen regimens could not be elucidated from the patient files as record keeping was poor in the majority of patients.

Conclusion

Fungal infections cause a high burden of disease in South Africa, driven largely by HIV, TB and poverty [27].

The diagnosis of invasive mold infections remains a challenge. In the current study, molds were found to cause serious infections, especially in at risk patients. However, molds are also common environmental organisms and therefore a common cause of contamination of

clinical specimens, thus highlighting the need for clinical correlation in the interpretation of these results. Despite treatment with appropriate antifungal agents, the associated mortality rate was still high.

We therefore recommend that a clinical consultation with an infectious diseases specialist and microbiologist should be considered for all patients with suspected invasive mold infections.

We believe this is an important study that contributes to the growing knowledge on the distribution, patient characteristics and outcomes of invasive mold infections, particularly in patients in the Free State, and lays the foundation for further research in the field of invasive mold infections.

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Appendix k

A case series: The distribution, patient characteristics, therapy and patient outcome in culture Positive invasive mold infections in HIV positive patients in a tertiary hospital in the Free State pro

by Bonita Vd Westhuizen

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A case series: The distribution, patient characteristics, therapy and patient outcome in culture positive invasive mold infections in HIV positive patients in a tertiary hospital in the Free State province, South Africa

Main author: Dr B van der Westhuizen
Supervisor: Prof Y Coovadia
Co-supervisor: Dr S Potgieter
Co-supervisor: Dr MS Abrahams

Key words:
HIV, CD4, Invasive mold infection, mold distribution, treatment, outcome

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Abstract

Introduction
Fungi, including molds, are increasingly recognized as important pathogens carrying a high morbidity and mortality in critically ill and immune compromised patients and our understanding of these diseases remain incomplete, largely due to the lack of surveillance data. This study aimed to better quantify the distribution, patient characteristics, risk factors, therapy and treatment outcome in culture positive invasive mold infections at Universitas Academic Hospital (UAH) in the Free State province, South Africa. This case series describes the HIV positive patients in this study.

Methods
All culture positive mold isolates cultured from sterile specimens were identified retrospectively from 1 July 2014 to 30 June 2017. Laboratory and clinical data were reviewed for those that met the inclusion criteria.

Results
The most common risk factors identified were HIV infection with a median CD4 of 88.5 cells/µl and hematological conditions. *Sporothrix schenkii* and *Bipolaris* species were the most common molds identified. The documented mortality was 14.2%.

Conclusions
This study contributes to the growing knowledge on the distribution, patient characteristics and outcomes of invasive mold infections, particularly in patients in the Free State, and lays the foundation for further research in the field of invasive mold infections.

Introduction

Fungi, including molds, [redacted] important pathogens in critically ill and immune compromised patients [1, 2]. Not only have these organisms assumed a greater role in human disease over the last 2 decades, but invasive fungal infections and in particular mold infections, are associated with significant morbidity and mortality [3, 4].

There is limited local data, regarding invasive mold infections especially in HIV positive patients, most likely due to diagnostic challenges.

We identified HIV as the most common risk factor (median CD4 88.5 cells/μl) a finding that has not been previously well documented in the literature. It should be noted that the background prevalence of HIV in the Free State is estimated at 5.1% as released in the midyear population estimates for 2018 by STATS SA (Statistics South Africa) [5].

Methods

With a retrospective review of laboratory and patient data, our study aimed to better quantify the distribution, patient characteristics, risk factors, therapy and treatment outcome in culture positive invasive mold infections at Universitas Academic Hospital (UAH) in the Free State province, South Africa between 1 July 2014 and 30 June 2017.

Case presentation

Patient 1

A 52-year-old male that presented with a history of crusted indurated plaques present over the forearms and face. He was newly diagnosed with HIV with a CD4 count of 13 cells/μl. *Sporothrix schenkii* was cultured on tissue samples and histology was positive for fungal elements. The patient received 14 days of intravenous ABD (amphothericin B deoxycholate) followed by oral itraconazole. He was down referred to his local primary care facility for palliative care. He never attended his follow up appointment.

Patient 2

A 52-year-old male presented with a nine month history of painful ulcerated large plaques on the face, limbs, trunk and back. He was HIV positive with a CD4 count of 65 cells/μl on antiretroviral therapy. *Sporothrix schenkii* was cultured on tissue samples and histological evaluation was suggestive of a deep fungal infection. He received 14 days of intravenous ABD followed by oral itraconazole. This patient responded well to therapy clinically as well as having documented negative follow up cultures.

Patient 3

A 42-year-old male presented with a thick confluent facial inflammatory plaque. He was newly diagnosed with HIV with a CD4 count of 21 cells/μl. *Sporothrix schenkii* was cultured on tissue samples and histological evaluation was suggestive of a deep fungal infection. He received 14 days of intravenous ABD followed by oral itraconazole. This patient responded well to therapy clinically.

Patient 4

A 44-year-old female patient presented with vulvar skin lesions. She was HIV positive on antiretroviral agents with a CD4 of 568 cells/μl. He was also known with metatstatic cervix carcinoma. A tissue biopsy was submitted for culture and histology. A *Bipolaris* species was cultured and histology was negative for fungal elements. In this patient the mold isolated was considered to be a contaminant and she did well despite not receiving any antifungal therapy.

Patient 5

A 28-year-old female patient presented with clinical features of sinusitis. She was a known HIV patient on antiretroviral therapy with a CD4 count of 484 cells/μl. She was also known with acute lymphocytic leukemia and was neutropenic on chemotherapy at the time of presentation. Middle turbinate tissue was submitted for culture and histology. A *Bipolaris* species was cultured and histology was suggestive of an invasive fungal infection. This patient received intravenous ABD as well as surgical debridement procedures (twice). She responded very well to the combined medical and surgical approach.

Patient 6

A 37-year-old male patient presented with a chronic fungal rhinosinusitis with maxillary sinus polyps. He was a known HIV positive patient on antiretroviral agents with a CD4 count of 328 cells/μl. The patient received endoscopic surgery to remove the polyps. Tissue was submitted for culture and histology. A *Bipolaris* species was cultured and histology was positive with numerous branching septate hyphae seen on fungal stains. The patient was treated with nasal saline and topical corticosteroids without receiving any antifungal therapy and had a favorable outcome. The use of antifungal agents in these patients' remains controversial. The currently available data as well as consensus guidelines do not support the use of antifungal agents in patients with chronic allergic rhinosinusitis.

Patient 7

A 36-year-old female patient presented with multiple firm nodules on the elbows, knees and distal lower legs and feet present for 6 months. She was HIV positive on antiretroviral therapy with a CD4 count of 142 cells/μl. Tissue biopsies were submitted for culture and histology. An *Aspergillus* species was cultured; however, histology was negative for fungal elements but suggestive of leprosy. This patient received therapy for leprosy as well as oral itraconazole. She responded well. The significance of the isolated mold in this case is questionable.

Patient 8

A 37-year-old male patient presented with lower limb skin lesions and one large, chronic, non-healing necrotic ulcer exposing his left foot tendons. He was HIV positive on antiretroviral therapy with a CD4 count of 291. Tissue specimens were submitted for culture and histology. An *Aspergillus* species was isolated. Histology of the skin lesions was suggestive of syphilis. The patient received a surgical above-knee-amputation and was treated for syphilis. He had a favorable outcome. It is uncertain whether the ulcer was due to the mold isolated or if it was merely a contaminant.

Patient 3

A 41-year-old male presented with a history of an acute onset cough, fever and dispnoea. He deteriorated rapidly and required intubation and ICU care. Imaging was suggestive of a fungal infection of the lung. The patient was newly diagnosed HIV positive with a CD4 count of 30 cells/μl. An endotracheal aspirate sample was sent for culture and an *Aspergillus* species was isolated. The patient was initiated on intravenous ABD combined with voriconazole but despite appropriate therapy, demised 3 days after diagnosis.

Patient 10

A 33-year-old female patient presented with diffuse cutaneous lesions. She was newly diagnosed HIV positive with a CD4 count of 1 cell/μl. She was also co-infected with Hepatitis B virus. Tissue samples were submitted for culture and histology. Histoplasma capsulatum was isolated and histology was also suggestive of this infection. She received 14 days of intravenous ABD followed by 2 years of oral itraconazole. She had a favorable outcome.

Patient 11

A 30-year-old male patient He presented with multiple papules, some with a necrotic centres. Other papules were confluent and flat on the face and some were asymptomatic. The face, trunk, arms and legs were all affected. He was newly diagnosed HIV positive with a CD4 count of 1 cell/μl. An *Emmonsia* species was isolated and fungal stains were positive on histology. He received 14 days of intravenous ABD followed by oral itraconazole. The patient was down referred to his local primary care facility to continue his treatment. He never attended his follow up appointment.

Patient 12

A 10-year-old male patient presented with subdural empyema and cerebral abscesses complicating a *Streptococcus pneumoniae* meningitis. He was HIV positive with a CD4 count of 198 cells/μl. A *Penicillium* species was cultured from the subdural empyema fluid collected during a surgical washout procedure. The patient was transferred to his local primary care facility before the fungal results was available. He never attended his follow up appointment. It is uncertain what the outcome of this patient or the significance of the isolated mold was.

Patient 13

A 45-year-old female patient with Burkitts lymphoma presented with diffuse skin lesions. She was newly diagnosed HIV positive with a CD4 count of 112 cells/μl. Tissue samples were submitted for culture only and histological evaluation was not requested. A *Cladosporium* species was isolated. In this patient the mold was considered to be a contaminant. She did not receive any antifungal therapy and had a favorable outcome.

Patient 14

A 32-year-old male patient presented with clinical features of rhino-orbital-cerebral mucormycosis. He was newly diagnosed with HIV with a CD4 count of 50 cells/μl. Palate, cheek and nasal tissue was submitted for culture and histology. *Saksenaea oblongispora* was isolated and confirmed by molecular testing. Histology was positive for an angio-invasive

fungal infection. He demised 3 days after the biopsies were submitted for evaluation. This patient was never started on any antifungal therapy and also did not receive any surgical intervention.

Discussion

In this cohort of patients, 14.3% (2/14) died, 64.3% (9/14) had a favorable outcome and 21.4% of patients were lost to follow up. These 3 patients were all started on antifungal therapy and then referred back to their primary facilities. None of them attended their follow up appointments. It is possible that these patients could have demised. If this assumption is true, it would increase the mortality to 35.71% (5/14) in the HIV positive group.

In the majority of patients 64.3% (9/14), the isolated mold was considered a significant pathogen. In these patients both culture and histology were positive. An interesting and clinically very important finding, is that 3 of these patients with very low CD4 cell counts of 65, 21, and 1 cells/µl respectively, responded very well to appropriate antifungal therapy and had a favorable outcome. Therefore, in this cohort, advanced disease did not equate to mortality.

The importance of source control is also highlighted by the fact that all patients in this cohort that received a surgical intervention had a good outcome.

Sporothrix schenkii affects all ages and genders but is usually associated with patients working in the agricultural sector (due to the main route of infection, traumatic inoculation, in these patients) and as expected, often males. The clinical presentation depends on the virulence of the organism, the size of inoculum and well as the patient's immune system. HIV-1 is associated with sporothrichosis with disseminated disease seen in the advanced stages if this infection [6, 7].

Bipolaris species forms part of the dematiaceous fungi, a heterogeneous group of fungi with [redacted]. Besides known to cause [redacted] [8]. No clear correlation between *Bipolaris* species and HIV is documented in literature.

Chronic pulmonary aspergillosis is now emerging as a complication due to lung damage caused by HIV related pulmonary tuberculosis. Previously, pulmonary aspergillosis was seen mainly secondary to AZT-related neutropenia, however, cases may occur in patients with advanced immunodeficiency due to HIV-1 infection [9].

Disseminated histoplasmosis is a known AIDS-defining disease and roughly 25% of patients with stage 4 HIV-1 infection presents with this infection in endemic areas [9].

Research done in South Africa, has recently shown an increase in patients presenting with an unmasking IRIS with *Emergomyces africanum* (formarly known as *Emmonsia* species) [10].

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A systematic review found that mucormycosis is a life-threatening infection in HIV infected individuals. This infection carries a very high mortality and is seen especially in patients also infected with other HIV-associated opportunistic infections and in those with very low CD4 cell counts. Predominant associated features were intravenous drug use, neutropenia and corticosteroid use [11]. *Sakseneae oblongispora* has recently been recognized as an emerging Zygomycete. This mucoratious mold has been described to cause severe skin infections after traumatic implantation or surgery mostly in previously healthy patients and occasionally in patients with hematological conditions [12]. To our knowledge, this is the first case of invasive *Saksanaea oblongispora* infection described in in the setting of HIV.

Conclusion

Important conclusions drawn from this cohort is that HIV infection is a common risk factor for invasive mold infections in South Africa. Advanced disease did not equate to mortality and the majority of patients responded well to appropriate therapy. The importance of source control was also demonstrated.

We believe it is an important study that contributes to the growing knowledge on the distribution, patient characteristics and outcomes of invasive mold infections, particularly in patients in the Free State, and lays the foundation for further research in the field of invasive mold infections.

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A case series: The distribution, patient characteristics, therapy and patient outcome in culture positive invasive mold infections in HIV positive patients in a tertiary hospital in the Free State pro

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Appendix l

A case series: The distribution, patient characteristics, therapy and patient outcome in culture positive invasive mold infections in HIV positive patients in a tertiary hospital in the Free State pro

by Bonita Vd Westhuizen

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A case series: The distribution, patient characteristics, therapy and patient outcome in culture positive invasive mold infections in HIV positive patients in a tertiary hospital in the Free State province, South Africa

Main author: Dr B van der Westhuizen
Supervisor: Prof Y Coovadia
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Co-supervisor: Dr MS Abrahams

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HIV, CD4, Invasive mold infection, mold distribution, treatment, outcome

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Abstract

Introduction
Fungi, including molds, are increasingly recognized as important pathogens carrying a high morbidity and mortality in critically ill and immune compromised patients and our understanding of these diseases remain incomplete, largely due to the lack of surveillance data. This study aimed to better quantify the distribution, patient characteristics, risk factors, therapy and treatment outcome in culture positive invasive mold infections at Universitas Academic Hospital (UAH) in the Free State province, South Africa. This case series describes the HIV positive patients in this study.

Methods
All culture positive mold isolates cultured from sterile specimens were identified retrospectively from 1 July 2014 to 30 June 2017. Laboratory and clinical data were reviewed for those that met the inclusion criteria.

Results
The most common risk factors identified were HIV infection with a median CD4 of 88.5 cells/µl and hematological conditions. *Sporothrix schenkii* and *Bipolaris* species were the most common molds identified. The documented mortality was 14.2%.

Conclusions
This study contributes to the growing knowledge on the distribution, patient characteristics and outcomes of invasive mold infections, particularly in patients in the Free State, and lays the foundation for further research in the field of invasive mold infections.

Introduction

Fungi, including molds, [redacted] important pathogens in critically ill and immune compromised patients [1, 2]. Not only have these organisms assumed a greater role in human disease over the last 2 decades, but invasive fungal infections and in particular mold infections, are associated with significant morbidity and mortality [3, 4].

There is limited local data, regarding invasive mold infections especially in HIV positive patients, most likely due to diagnostic challenges.

We identified HIV as the most common risk factor (median CD4 88.5 cells/μl) a finding that has not been previously well documented in the literature. It should be noted that the background prevalence of HIV in the Free State is estimated at 5.1% as released in the midyear population estimates for 2018 by STATS SA (Statistics South Africa) [5].

Methods

With a retrospective review of laboratory and patient data, our study aimed to better quantify the distribution, patient characteristics, risk factors, therapy and treatment outcome in culture positive invasive mold infections at Universitas Academic Hospital (UAH) in the Free State province, South Africa between 1 July 2014 and 30 June 2017.

Case presentation

Patient 1

A 52-year-old male that presented with a history of crusted indurated plaques present over the forearms and face. He was newly diagnosed with HIV with a CD4 count of 13 cells/μl. *Sporothrix schenkii* was cultured on tissue samples and histology was positive for fungal elements. The patient received 14 days of intravenous ABD (amphothericin B deoxycholate) followed by oral itraconazole. He was down referred to his local primary care facility for palliative care. He never attended his follow up appointment.

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