The University of the Witwatersrand

Faculty of Health Sciences

Invasive Fungal Disease in Children with Cancer: a 10 year analysis from a Paediatric Oncology Centre in Soweto, South Africa

By

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Declaration

I Abigail Marriott Keene declare that this Research Report is my own, unaided work. It is being submitted for the Degree of Masters of Medicine in Paediatrics at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

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(Signature of the candidate)

_____ day of _____ 2024 in _____

Dedication

I dedicate this work to my family.

My husband, Stephen, for being my person.

My parents, Tony and Penny, for being my inspiration.

Presentations and Publications

I will present the findings of this study at the University of the Witwatersrand Paediatric Research Day on the 7th of December 2023.

Abstract

Background

Invasive fungal diseases (IFD) pose a significant threat to immunocompromised children, including those with cancer. There is a paucity of data on IFD in children and adolescents undergoing treatment for cancer in low-middle income countries, including South Africa.

Methods

We reviewed the clinical and laboratory characteristics, and factors associated with microbiologically-confirmed IFD in children and adolescents treated at Chris Hani Baragwanath Academic Hospital (CHBAH) Paediatric Oncology Unit from 01 January 2011 through 31 December 2020.

Results

During the 10-year study period, 211 microbiologically-confirmed IFD episodes occurred in 174 children and adolescents treated in the Oncology Unit. There were sustained reductions in the number of microbiologically-confirmed IFD episodes from 2018, onwards. Most of the episodes (179, 84.8%) occurred in children and adolescents with haematological malignancies (n=93) or solid tumours (n=86). *Candida albicans* (n=71) and *C. parapsilosis* (n=66) were isolated in 64.9% of the episodes, other Candida spp in 23.2% (n=49), Aspergillus spp in 1.9% (n=4) and other fungi in 10.0% (n=21) of the IFD episodes. *Candida albicans* and *C. parapsilosis* susceptibility to fluconazole remained >80% throughout the study period. In 197 episodes with known clinical outcome, 21 (10.7%) resulted in death. In multivariable Poisson regression analysis, severe malnutrition (adjusted risk ratio (aRR), 2.904; 95% confidence interval (CI) 1.165-6.918) and severe neutropenia (aRR, 4.483; 95%CI, 1.835-12.141) were independently associated with death following the IFD episode.

Conclusions

Microbiologically-confirmed IFD is an uncommon complication of treatment in children with cancer, although associated with high crude case fatality rates. Severe malnutrition and severe neutropenia are independently associated with death. Optimisation of infection prevention and control measures, and fungal prophylaxis targeted at those most likely to die, may impact on survival outcomes in patients with IFD.

Acknowledgements

I would like to give special thanks to the patients in the CHBAH Paediatric Oncology Unit, from whom I have learned so much. I hope this research manages to help them in the future.

I am so grateful to my supervisors: Prof Gita Naidu, who has been a mentor and role model for me from before this process had even started, thank you for the unwavering support. Prof David Moore who sacrificed so much of his time to ensure we had something to be proud of to hand in. I am also very thankful for the help that I received from Dr Wadula in this endeavour.

I also want to acknowledge the great impact my sister, Claire, has had on my academic journey so far. I look up to you and appreciate you so much.

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Nomenclature

ALL	acute lymphoblastic leukaemia
AML	acute myeloid leukaemia
ANC	absolute neutrophil count
ART	antiretroviral therapy
BDG	β-D-Glucan
CHBAH	Chris Hani Baragwanath Academic Hospital
CLWH	children living with HIV
CRP	C-reactive protein
GM	galactomannan
HIV	human immunodeficiency virus type-1
HSCT	haematopoietic stem cell transplantation
IFD	invasive fungal disease
LASSO	least absolute shrinkage and selection operator
LMIC	low- and middle-income countries
MAM	moderate acute malnutrition
MUAC	mid-upper arm circumference
NHLS	National Health Laboratory Service
PCR	polymerase chain reaction
PCT	procalcitonin
TB	tuberculosis
TPN	total parenteral nutrition
SAM	severe acute malnutrition
WHO	World Health Organisation

1 LITERATURE REVIEW

1.1 Background and rationale for study

Invasive fungal diseases (IFD) are opportunistic infections, rare in patients with a normally functioning immune system[1]. However, they pose a significant threat to immunocompromised patients such as children with cancer[2], amongst whom the most common cause of treatment-related morbidity and mortality are infections[3]. There has been a trend of increased survival in paediatric cancer patients owing to improvements in cancer treatment regimens as well as antimicrobial therapy[1,3]. Advancements in chemotherapy, surgical techniques, radiotherapy and haematopoietic stem cell transplantation predispose cancer patients to longer and more severe episodes of immunosuppression with periods of profound, prolonged neutropenia. This places them at an increased risk for infections, including IFD, and increases exposure to broad-spectrum antibiotics, which also predispose to IFD[1–3]. While IFD cause significant morbidity and mortality[2], there is a paucity of studies published on the topic in low- and middle-income countries (LMIC), including South Africa. Mortality from IFD varies from 20-70% depending on the degree of immunosuppression, underlying co-morbidities, severity of infection, and time to diagnosis and initiation of appropriate antifungal therapy[4]. The ability to diagnose and treat IFD is a vital component in the successful treatment of children with cancer.

1.2 Fungal Diseases

Fungal yeasts and moulds cause a variety of clinical presentations as a result of the interaction between the pathogen and the human host. The host immune response, underlying malignant disease and its treatment, and co-morbidities impact on the presentation and severity of IFD in children with cancer[3,5]. Signs and symptoms of IFD may be absent until such time as there is immune reconstitution in severely immunocompromised hosts[5]. It is therefore imperative to have a strong clinical suspicion of IFD, and to actively look for evidence of IFD in any high-risk patient, especially in those with persistent fever despite prolonged broad-spectrum

antibiotic use (more than 96 hours) and in those with prolonged neutropenia (of at least ten days)[6]. Rosen et al[2] described IFD as the isolation of a fungus from a bodily fluid or tissue that is normally sterile, with accompanying constitutional symptoms (for example, fever). There may also be pathological or radiographic evidence of invasive infection despite absence of growth on cultures of clinical specimens[2]. IFD are classified as proven, possible or probable based on the certainty of the diagnosis[1].

1.2.1 Proven IFD

Criteria for proven mould infection include the histopathological, cytopathological or microscopic finding of hyphae in association with tissue damage on examination of a sterile tissue specimen, or a blood culture that grows a mould[1]. A diagnosis of proven yeast infection is established by histopathological, cytopathological or microscopic findings of yeast cells on sterile specimens, or by demonstrating yeast on a culture of a sterile site with clinical or radiological findings consistent with infection[1]. Proven *Cryptococcus neoformans* infection is demonstrated by the presence of cryptococcal antigen in blood or cerebrospinal fluid[1].Cryptococcal antigen can remain positive in blood or cerebrospinal fluid for months to years following the diagnosis and treatment of the fungus. In the absence of new signs or symptoms following the successful treatment, a positive cryptococcal antigen is not an indication of a relapse in these patients[7].

1.2.2 Possible or probable IFD

Possible or probable fungal infection is diagnosed based on specific host factors, with the presence of clinical criteria consistent with IFD as well as mycological evidence of infection, but where blood cultures remain negative for fungal pathogens[1]. Host factors include prolonged, profound neutropenia, T-cell immunosuppressive therapy or an inherited immunodeficiency, use of corticosteroids, haematopoietic stem cell transplant, or frequent and prolonged use of broad-spectrum antibiotics. At least one of the aforementioned factors must be present with clinical criteria suggestive of IFD. Clinical features of IFD include radiographic changes

in the lower respiratory tract, sinuses or central nervous system, or evidence of tracheobronchitis on bronchoscopy. There should also be evidence of mould in respiratory tract samples, e.g. sputum or bronchoalveolar lavage, or positive non-culture or indirect tests such as galactomannan (GM) or β-D-glucan (BDG)[1].

1.2.3 Fungal species implicated in IFD

The most common fungal pathogens in paediatric oncology patients are Candida spp and Aspergillus spp[1,2].

1.2.3.1 Yeast – Candida spp

Candida spp are responsible for the majority of IFD in paediatric cancer patients[4], causing up to 69% of IFD in this population in a study by Rosen et al in 2005[2]. Candida spp are part of the normal gastrointestinal flora and, while they can cause localised or superficial infections of the mucous membranes in the immunocompetent host, they can cause disseminated disease in the immunocompromised patient[3]. Yeast cells enter the bloodstream at points of mucosal or cutaneous breakdown such as at central venous catheter insertion sites or at sites of chemotherapy-induced mucosal disruption[1,3]. The most common presentation is fever refractory to broad-spectrum antibiotic therapy[4]. Severe sepsis and shock occur in about 30% of patients[4]. Up to half of all patients with proven invasive candidiasis will have a negative blood culture due to the low sensitivity of the blood cultures for fungi[5]. Automated blood culture systems, such as the BacT/Alert System, have a sensitivity of about 50% for invasive candidiasis[4]. Candidaemia occurs when there is a positive blood culture in the absence of deep-seated infection. Candida infections can disseminate to the central nervous system, eyes, liver, spleen, lungs and kidneys. Disseminated or deep-seated fungal infection (typically hepatosplenic) may also occur in the absence of candidaemia or a positive culture[8].

1.2.3.2 Moulds – Aspergillus spp

Invasive aspergillosis has a predilection for both the upper and lower respiratory tracts. In

primary invasive pulmonary aspergillosis, approximately 50% of patients have signs and symptoms of a respiratory tract infection, while up to 62% of patients with sino-nasal disease can remain asymptomatic[5]. It is likely that invasive aspergillosis is under-diagnosed and under-treated in children with cancer. Aspergillosis can also present as a skin lesion, which is more common in children than in adults. Cutaneous aspergillosis can occur as a result of direct spore inoculation, or from haematogenous spread as part of disseminated disease[1,5].

1.2.4 Fungal disease diagnosis

The gold standard in diagnosis of IFD is tissue histopathology and culture[9]. Tissue biopsies are not always feasible in cancer patients due to the presence of coagulopathies, neutropenia and clinical instability[4]. Blood cultures are only capable of detecting fungal cells that are still viable. A negative blood culture may occur due to lack of viable fungal cells in the bloodstream, an insufficient concentration of viable fungal cells, intermittent release of viable fungal cells into the circulation, treatment with antifungal therapy prior to specimen collection, or suboptimal sample volume[8]. The exact sensitivity of fungal blood cultures ranges with different studies, but Clancy et al[8] described that in autopsy-proven invasive candidiasis in adults, the sensitivity of ante-mortem blood cultures ranged from 21% to 71%[8]. Fungal cultures also have a median time to positivity of two to three days, which can further delay appropriate treatment and worsen patient outcomes[8].

It is important to review adjunctive methods of screening for and diagnosing IFD. These include 'non-culture' biomarkers that are fungal or pathogen-derived, such as BDG, GM (specifically for Aspergillus spp), cryptococcal antigen and polymerase chain reaction (PCR) assays identifying fungal DNA[1,9]. These 'non-culture' tests seem to exhibit high negative predictive values (up to 97%) with low positive predictive values (8-23%), which limits their use in diagnosing IFD. More studies need to be done on these adjunctive diagnostic methods in paediatrics[1], however current recommendations by the International Paediatric Febrile Neutropenia Guidelines do not advocate their routine use in diagnosing IFD in children[1,10].

1.3 Risk factors for IFD

There are established risk factors for the high incidence of IFD in children with cancer, including the underlying cancer diagnosis and therapy-induced complications[5,6,11]. High-risk malignancies and treatments include acute myeloid leukaemia (AML), high risk acute lymphoblastic leukaemia (ALL) including relapsed disease, highly myelosuppressive chemotherapy, and allogeneic haematopoietic stem cell transplantation (HSCT)[6]. Therapy-induced complications include the presence of neutropenia. Neutropenia is considered to be profound with an absolute neutrophil count (ANC) of less than 500 cells/uL, and prolonged if lasting for seven to ten days[5,11]. Other recognised risk factors include the use of total parenteral nutrition (TPN), chemotherapy agents and other immunosuppressive drugs used during treatment such as high dose corticosteroids[5]. Corticosteroids at an equivalent dose of prednisone of 2 mg/kg/day confer a high risk for the development of IFD, owing to steroid-induced impairment of phagocytosis, neutrophil function and the humoral immune response[4]. Chemotherapy-induced mucosal injury, and intravenous, central venous and indwelling catheters are recognised risk factors for IFD[5,6,11]. Concurrent or preceding bacterial infection requiring the use of broad-spectrum antibiotics also predispose to IFD in immunocompromised patients[5].

In the South African setting, other co-morbidities, including malnutrition, human immunodeficiency virus type-1 (HIV) infection and tuberculosis (TB), place children with cancer at risk for developing IFD.

1.3.1 Malnutrition

Eighty per cent of children with cancer live in LMIC[12,13]. Malnutrition is endemic in LMIC with a prevalence of 50% to 70%[13]. In a study in Pretoria in 2008, 22% of paediatric cancer patients were wasted at the time of their diagnosis, and 24% were underweight[14]. Malnutrition has significant consequences in the treatment and survival of malignancies. There is a well-established connection between malnutrition and increased risk of infection,[15] because malnutrition is associated with depressed cell-mediated immunity[16], decreased antibody

affinity[16] and higher frequency and longer duration of neutropenia in cancer patients,[14,16] as well as more severe neutropenia (lower nadir of the ANC)[12,15]. Malnutrition is also associated with higher chemotherapy-associated toxicity rates[13] and reduced tolerance to therapy resulting in treatment delays, longer hospital stays and, occasionally, abandonment of care[14] with increased morbidity and mortality in these patients[12].

Malnutrition in children with cancer is multifactorial and is associated with socio-economic status, maternal educational status[17], the type of cancer and the treatment of the cancer[18]. Cancer and chemotherapy induce a catabolic state, which leads to a reduction in lean body mass, and malnutrition. Treatment-associated factors such as nausea and vomiting, oral mucositis, poor appetite and food aversion play a significant role in reduced nutrient intake and subsequent malnutrition[18].

Malnutrition is therefore an important co-morbidity to identify as it has significant negative consequences on survival rates, despite being treatable. Malnutrition is particularly important with regards to IFD, as it causes a longer duration of and more frequent episodes of neutropenia, which is the most significant and commonly identified risk factor for IFD[19]. A study conducted in Malawi showed that patients with Burkitt Lymphoma who had profound and prolonged neutropenia during their treatment, as well as patients with treatment-related deaths, had malnutrition at the time of their oncological diagnosis[15].

1.3.2 HIV

In 2022, there were 230,000 children (between 0 - 14 years old) with HIV living in South Africa, of which 54% were on antiretroviral treatment (ART)[20]. The complications of HIV have been well described, including an increased predisposition for opportunistic infections, including IFD, as a consequence of HIV-induced immunodeficiency and immune dysfunction[21]. HIV is therefore an independent risk factor for the development of IFD.

1.3.3 Tuberculosis

South Africa has a high incidence of TB. In 2020, 22 960 cases were reported in children younger than 15 years old.[22] Children with cancer are immunocompromised owing to the disease itself as well as the treatment they receive and are therefore at high risk of progressing from *Mycobacterium tuberculosis* infection to TB disease. In a study conducted in Johannesburg which evaluated 169 children with various malignancies, five (2.9%) were diagnosed with TB prior to treatment of the cancer, and a further 34 (20.7%) were diagnosed and treated for TB following initiation of cancer treatment[23]. Furthermore, subsequent to the diagnosis and treatment of TB, there were higher rates of sepsis with prolonged neutropaenia[23]. This may put them at a higher risk for developing IFD.

1.4 Treatment of IFD

Treatment of IFD should include not only appropriate antifungal agents, but also surgical resection if warranted, and reconstitution of the immune system[1]. While appropriate antifungal drugs are the mainstay of treatment, they can be inadequate if used alone, as there is poor penetration into areas of tissue necrosis caused by invasive infection. When surgical resection is combined with antifungal therapy, the odds of successful treatment increase[1]. Immune reconstitution is key in the treatment of paediatric oncology patients with IFD[1]. Therefore, immunosuppressive medications including chemotherapy and corticosteroids should be minimised if possible until the IFD episode resolves. Granulocyte colony-stimulating factor may be used as adjunctive therapy to promote neutrophil recovery in IFD patients with low neutrophil counts[1].

There are three classes of antifungal agents commonly used in children with cancer and fungal infection: polyenes, triazoles and echinocandins[3].

1.4.1 Polyenes

Polyenes include amphotericin B, an antifungal with a broad spectrum of activity, effective against yeasts and moulds. Amphotericin B has good penetration into most tissues, is widely available and affordable, and is therefore the standard antifungal drug in most LMIC[3]. Currently, in South Africa, amphotericin B is available only as an intravenous formulation for IFD, and as a lozenge for localised oral and perioral infections. Oral formulations have been developed abroad, but are not yet available in this setting[24]. The drug has recognised side effects such as renal toxicity, electrolyte imbalances (including hypokalaemia and hypomagnesaemia), and acute allergic reactions (which may manifest as fever, chills, hypotension, nausea and vomiting)[3].

1.4.2 Triazoles

Triazoles include fluconazole and voriconazole. Fluconazole is commonly used in children with cancer for prophylaxis against IFD. It is also used to treat mucosal infections, and as step-down or continuation therapy for invasive candidiasis following initial therapy with a polyene or echinocandin[3]. Fluconazole is relatively inexpensive and is widely available in LMIC, however it does not have suitable activity against moulds such as Aspergillus spp, and has drug interactions with many other medications, including rifampicin, anti-epileptic drugs, protein pump inhibitors and macrolides[3].

1.4.3 Echinocandins

Echinocandins include micafungin, caspofungin and anidulafungin. Echinocandins disrupt the fungal cell wall and are fungicidal against Candida spp and fungistatic against Aspergillus spp[3]. This class of drug is recommended for first line use in invasive Candida spp infections, and as a second line or adjunctive therapy for aspergillosis. They have no activity against *Cryptococcus neoformans*, and have poor central nervous system penetration[3].

1.5 Justification for this study

IFD is a serious, significant problem in children with cancer. Few studies have been dedicated to describing the burden of disease and epidemiology of IFD in children with cancer in LMIC. Risk factors, diagnostic methods and treatment in high income settings are well described in the literature. However, in LMIC settings there may be additional risk factors which predispose towards the development of IFD, as well as barriers to diagnosis and treatment of IFD. Children with cancer are just as susceptible to IFD as adults with cancer, however the two groups are significantly different with regards to their physiology, co-morbidities, and their tolerance to treatment. Extrapolation of findings and recommendations from adult studies are not always appropriate in the management of children with cancer[4]. In order to optimise the care of children with cancer, studies designed to evaluate risk factors and characteristics of IFD are needed to inform preventive, diagnostic, and treatment approaches.

1.6 Aims and objectives

1.6.1 Study Aim

To describe the epidemiology of IFD in children with cancer, diagnostic parameters, antifungal treatment and patient outcomes in a LMIC setting.

1.6.2 Objectives

- To describe the prevalence of IFD in children with cancer treated at the Chris Hani Baragwanath Academic Hospital (CHBAH) Paediatric Oncology Unit;
- To describe the most commonly cultured fungal species in the unit;
- To assess the antifungal susceptibility profiles of the fungi cultured, and to establish whether the antifungal susceptibility profiles have changed over time;
- To describe the types of malignancy most commonly associated with IFD in this setting;
- To evaluate the ANC of the patients at the time of the IFD diagnosis;
- To assess the adjunctive diagnostic methods used in the Unit;

- To describe the antifungal treatment used in the Unit;
- To evaluate the prevalence of malnutrition in children with cancer who develop IFD, and to compare the outcomes of IFD in children with malnutrition to those in children with normal nutritional status;
- To evaluate the role of HIV in IFD in children with cancer, including whether HIV-infected children with cancer are more susceptible to IFD than those who are HIV-uninfected, and their outcomes;
- To assess the overall outcomes of paediatric cancer patients with IFD.

2 MANUSCRIPT IN SUBMISSIBLE FORMAT

Abstract

Background

Invasive fungal diseases (IFD) pose a significant threat to immunocompromised children, including those with cancer. There is a paucity of data on IFD in children and adolescents undergoing treatment for cancer in low-middle income countries, including South Africa.

Methods

We reviewed the clinical and laboratory characteristics, and factors associated with microbiologically-confirmed IFD in children and adolescents treated at Chris Hani Baragwanath Academic Hospital (CHBAH) Paediatric Oncology Unit from 01 January 2011 through 31 December 2020.

Results

During the 10-year study period, 211 microbiologically-confirmed IFD episodes occurred in 174 children and adolescents treated in the Oncology Unit. There were sustained reductions in the number of microbiologically-confirmed IFD episodes from 2018, onwards. Most of the episodes (179, 84.8%) occurred in children and adolescents with haematological malignancies (n=93) or solid tumours (n=86). *Candida albicans* (n=71) and *C. parapsilosis* (n=66) were isolated in 64.9% of the episodes, other Candida spp in 23.2% (n=49), Aspergillus spp in 1.9% (n=4) and other fungi in 10.0% (n=21) of the IFD episodes. *Candida albicans* and *C. parapsilosis* susceptibility to fluconazole remained >80% throughout the study period. In 197 episodes with known clinical outcome, 21 (10.7%) resulted in death. In multivariable Poisson regression analysis, severe malnutrition (adjusted risk ratio (aRR), 2.904; 95% confidence interval (CI) 1.165-6.918) and severe neutropenia (aRR, 4.483; 95%CI, 1.835-12.141) were independently associated with death following the IFD episode.

Conclusions

Microbiologically-confirmed IFD is an uncommon complication of treatment in children with cancer, although associated with high crude case fatality rates. Severe malnutrition and severe neutropenia are independently associated with death. Optimisation of infection prevention and control measures, and fungal prophylaxis targeted at those most likely to die, may impact on survival outcomes in patients with IFD.

Introduction

Invasive fungal diseases (IFD) are opportunistic infections that are rare in patients with a normally functioning immune system[1]; however, they pose a significant threat to immunocompromised patients, including children with cancer[2]. While IFD cause significant morbidity and mortality in this population[2], there is a paucity of studies published on the topic in low- and middle-income countries (LMIC). IFD is also associated with high rates of mortality (20-70%) in children with cancer[4].

The fungal yeasts and moulds most implicated in IFD are Candida spp and Aspergillus spp respectively[1,2]. Candida spp cause up to 69% of IFD in children with cancer[2]. Invasive aspergillosis may present as a skin lesion or more commonly sinonasal and respiratory disease[5]. These pathogens cause a variety of clinical presentations as a result of the interaction between the fungal pathogen and the human host, with host immune suppression, type of cancer, treatment regimens and comorbidities influencing the clinical spectrum and severity of disease[3,5].

IFD may be classified as proven, possible or probable based on the certainty of the diagnosis[1]. Proven IFD is diagnosed based on the histopathological, cytopathological or microscopic confirmation of a yeast or mould from a sterile site with clinical or radiological signs consistent with infection[1]. Possible or probable IFD is defined by having host risk factors for IFD along with evidence of fungal infection, but with no growth of a fungus on culture[1]. Non-culture or indirect tests such as galactomannan (GM) or β-D-glucan (BDG) may assist in establishing a diagnosis of IFD in cases in which cultures remain sterile[1]. Automated blood culture systems have a sensitivity of about 50% for invasive candidiasis[4]. More studies are needed on these biomarkers in paediatrics due to their low positive predictive values (8-23%), and despite their high negative predictive values (up to 97%)[1] they are not recommended by the International Paediatric Febrile Neutropenia Guidelines in diagnosis IFD in children[1,10].

It is likely that IFD is under-diagnosed and undertreated in children with cancer[4,8]. In this study, we reviewed the clinical characteristics of children and adolescents with cancer that

were treated at an academic centre in Johannesburg, South Africa, over a 10-year period. We aimed to describe the spectrum of fungal isolates, the disease manifestations and associated clinical characteristics of patients with proven IFD.

Methods

We conducted a retrospective record review of paediatric patients who were diagnosed with proven IFD while receiving treatment for cancer at the Paediatric Oncology Unit at Chris Hani Baragwanath Academic Hospital (CHBAH) from 01 January 2011 to 31 December 2020. Children and adolescents were included in the study if they were aged 0 to 19 years, and had cultures positive for a fungus from any normally sterile site (proven IFD cases only). We considered cases in which the same fungal species was cultured in serial cultures during the same septic episode as being a single episode of IFD.

Children and adolescents with presumed or probable IFD based on non-culture tests only were not included in this study. Other exclusion criteria were children with non-cancer diagnoses, e.g. aplastic anaemia, and patients with incomplete clinical records.

Data collection

Data were collected, with permission, from patient records. Patients fulfilling the inclusion criteria were identified using an electronic database. Data extracted from clinical records included demographic data (age, sex, socio-economic status), HIV infection status, anthropometry, type of malignancy and intensity of chemotherapy, exposure to high dose corticosteroid therapy, presence of indwelling lines or urinary catheters, use of total parenteral nutrition (TPN), abdominal surgery, and duration of hospitalisation and antibiotic therapy prior to onset of the IFD episode. Laboratory indices included full blood count, differential count, absolute neutrophil count (ANC) at the time of proven IFD diagnosis, fungal culture specimen type and fungal isolate, antifungal susceptibility of the isolate, and markers of infection, including BDG, GM, C-reactive protein (CRP), and/or procalcitonin (PCT) at the time of IFD diagnosis. Antifungal therapy, duration of therapy and patient outcomes were

also recorded.

The level of treatment intensity patients were receiving was classified as low, moderate or high. The classification was taken from an article by Naidu et al in 2020, published in The Pediatric Infectious Disease Journal[23]. Treatment was categorized as: 1. Low: localised solid tumours. 2. Moderate: metastatic solid tumours, medium-risk ALL and Hodgkin lymphoma. 3. High: high-risk ALL, AML and non-Hodgkin lymphoma.

Data analysis

Categorical or binary data are presented as proportions (i.e. percentages), and analysed using the Chi-square test, or Fisher's exact test, as appropriate. For continuous variables, the difference in means between groups with normally distributed data were analysed using the Student's t-test. Non-parametric tests were used to compare the medians of non-normally distributed continuous data. Receiver operating characteristic curves were generated to define cut-off levels of biomarkers used to diagnose IFD. Poisson regression was used to evaluate clinical parameters that were associated with death as the outcome of interest. Variable selection for the multivariable models was performed using least absolute shrinkage and selection operator (LASSO) regression. P-values of <0.05 were considered statistically significant. Analyses were conducted using R version 4.3.1.[25]

Ethical considerations

The study was approved by the Human Research Ethics Committee of the University of the Witwatersrand (clearance number: M210701) and the Ethics Committee of the NHLS (clearance number: PR2119246).

Results

From 28 January 2011 to 20 December 2020, 211 IFD episodes occurred in 174 children and adolescents that were treated for cancer at the CHBAH Paediatric Oncology Unit (Figure 2.1). One hundred, twenty-three of the IFD episodes occurred in children with haematolog-

ical malignancies, 86 occurred in children with solid organ malignancies, and two occurred in children with other diagnoses (Table 2.1). Half of the episodes occurred in males (106 episodes) and 9.4% (20 episodes) occurred in CLWH.

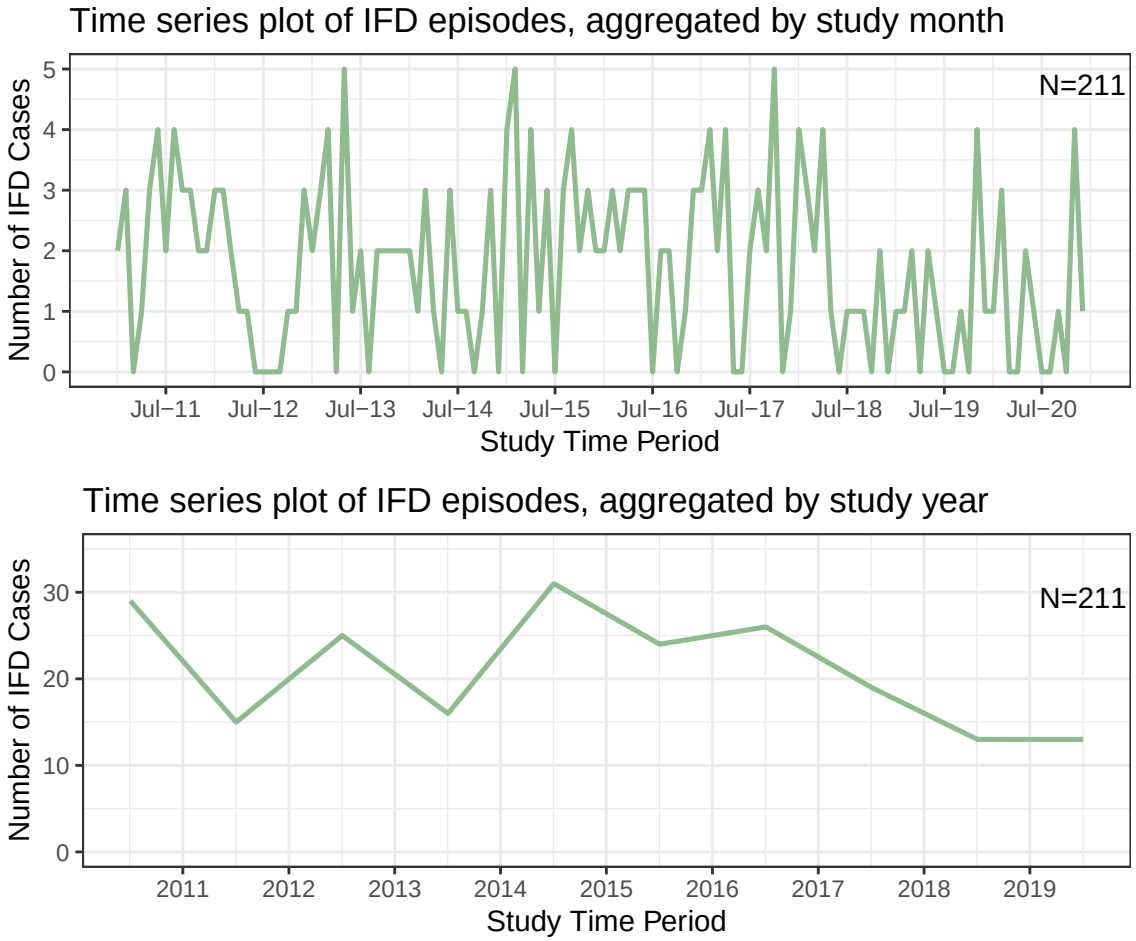

Figure 2.1: Timeseries plots of the number of invasive fungal disease episodes during the study time period

Baseline characteristics

The median age at cancer diagnosis was 60.0 months (interquartile range (IQR) 26.3 to 104.0 months), while the median age at IFD diagnosis was 65.6 months (IQR 30.0 to 109.6 months). There was a median time of 3.7 months from cancer diagnosis to diagnosis of IFD (IQR 1.6 to 7.4 months) (Table 2.1).

Ninety-three percent of IFD episodes occurred with receipt of high or moderate intensity chemotherapy (Table 2.1). Candida spp were the most prevalent fungal isolates comprising

88% of the total. Aspergillus spp contributed a very small percentage (4 isolates; 2%). There were significant differences in patient characteristics, based on a stratification according to type of cancer (Table 2.1). Those with lymphoma were significantly older (median age 109.8

Table 2.1: Clinical characteristics of IFD episodes, stratified by cancer classification

	Overall	Leukaemia	Lymphoma	Solid	Other	p
n	211	93	30	86	2	
Male (%)	106 (50.2)	44 (47.3)	23 (76.7)	38 (44.2)	1 (50.0)	0.019
Age (months) at cancer Dx [IQR]	60.35 [26.97, 104.35]	64.69 [28.03, 94.87]	109.83 [67.71, 173.64]	41.52 [15.61, 76.08]	21.93 [15.18, 28.68]	<0.001
Age (months) at IFD [IQR]	65.59 [30.00, 109.66]	69.85 [35.76, 102.43]	124.61 [71.57, 176.24]	46.45 [24.63, 83.18]	30.73 [26.00, 35.47]	<0.001
Time from Dx to IFD onset (months) [IQR]	3.72 [1.65, 7.47]	3.65 [1.83, 7.35]	2.94 [1.39, 6.66]	3.93 [1.48, 7.89]	8.78 [6.79, 10.78]	0.599
HIV Positive (%)	20 (9.5)	3 (3.2)	15 (50.0)	2 (2.3)	0 (0.0)	<0.001
Mean WFA Z-Score (SD)	-0.88 (1.50)	-0.34 (1.44)	-1.52 (1.51)	-1.31 (1.37)	-0.44 (2.05)	<0.001
Median HFA Z-Score [IQR]	-0.92 [-2.00, -0.06]	-0.49 [-1.39, 0.52]	-1.89 [-2.73, -0.82]	-1.10 [-2.05, -0.11]	-0.90 [-1.41, -0.40]	0.002
Mean WFH Z-Score (SD)	-0.72 (1.66)	-0.28 (1.74)	-1.11 (1.51)	-1.04 (1.56)	0.09 (1.89)	0.075
Median BMI Z-Score [IQR]	-0.76 [-1.89, 0.39]	-0.26 [-1.42, 0.79]	-1.29 [-2.24, -0.27]	-0.90 [-2.27, -0.02]	0.21 [-0.38, 0.80]	0.011
Treatment intensity						<0.001
Low	15 (7.1)	0 (0.0)	0 (0.0)	15 (17.4)	0 (0.0)	
Moderate	83 (39.3)	6 (6.5)	4 (13.3)	71 (82.6)	2 (100.0)	
High	113 (53.6)	87 (93.5)	26 (86.7)	0 (0.0)	0 (0.0)	
ANC Category (%)						0.063
Normal or mild	107 (51.9)	45 (48.4)	13 (43.3)	48 (59.3)	1 (50.0)	
Moderate or severe	39 (18.9)	17 (18.3)	3 (10.0)	19 (23.5)	0 (0.0)	
Profound	60 (29.1)	31 (33.3)	14 (46.7)	14 (17.3)	1 (50.0)	
Prolonged profound ANC (%)	34 (20.4)	21 (27.6)	7 (25.9)	5 (8.1)	1 (50.0)	0.020
Fungal group (%)						0.307
Candida albicans	71 (33.6)	30 (32.3)	11 (36.7)	30 (34.9)	0 (0.0)	
Candida parapsilosis	66 (31.3)	28 (30.1)	8 (26.7)	28 (32.6)	2 (100.0)	
Candida krusei	16 (7.6)	9 (9.7)	5 (16.7)	2 (2.3)	0 (0.0)	
Candida tropicalis	6 (2.8)	5 (5.4)	1 (3.3)	0 (0.0)	0 (0.0)	
Other Candida spp	27 (12.8)	12 (12.9)	2 (6.7)	13 (15.1)	0 (0.0)	
Aspergillus spp	4 (1.9)	2 (2.2)	1 (3.3)	1 (1.2)	0 (0.0)	
Other fungi	21 (10.0)	7 (7.5)	2 (6.7)	12 (14.0)	0 (0.0)	
Died (%)	21 (10.7)	8 (9.4)	7 (25.0)	6 (7.3)	0 (0.0)	0.061

Note:
HIV = human immunodeficiency virus type 1; HFA = height-for-age; IFD = invasive fungal disease; IQR = interquartile range; WFA = weight-for-age; WFH = weight-for-height. P-values are derived from tests that compare across malignancy classification: Chi-square test or Fisher's exact test, as appropriate, for categorical variables; Student t test for comparison of means; Kruskal-Wallis test for comparison of medians. The 'Other' cancer diagnoses were Langerhans cell histiocytosis in two patients.

months; IQR, 67.7 to 173.6 months) than children and adolescents with other types of cancer. Furthermore, 50.0% (15/30) of the lymphoma patients were HIV infected (Table 2.1). Children and adolescents with lymphoma also had significantly lower weight-for-age (Median -1.52) and height-for-age Z-scores (Median -1.89) than those in other groups, and had the lowest BMI-for-age Z-scores (Median -1.29) (Table 2.1).

IFD episodes in the lymphoma group occurred mostly in male patients (76.7%), and IFD diagnoses occurred a median of 2.9 months (IQR 1.39 to 6.66) after cancer diagnosis (Table 2.1).

The median age at which IFD episodes occurred among the children with solid tumours was 41.5 months (IQR 24.63 to 83.18), with the IFD occurring on average at 3.9 months (IQR 1.48 to 7.78) into the cancer diagnosis. None of the children with solid tumours were subjected to high intensity chemotherapy, and only two episodes occurred in CLWH (2.3%).

Two children with Langerhans cell histiocytosis developed one IFD episode each (Table 2.1). Both were on moderate intensity chemotherapy, and the IFD episodes occurred at a median of 8.8 months (IQR 6.79 to 10.78) after diagnosis of the condition (Table 2.1). Neither child with Langerhans cell histiocytosis died (Table 2.1).

Of the 206 patients with an available ANC result, 107 (51.9%) had either a normal result or were mildly neutropenic. The rest of the patients (99/206, 48.1%) had neutropenia that was moderate, severe or profound. Of the 60 patients with a profound neutropenia, 34 (56.7%) had prolonged neutropenia.

IFD episodes in children with malnutrition, TB and CLWH

There were 23 children with severe malnutrition, with a male predominance of 73.9%. Children and adolescents with malnutrition were diagnosed with cancer at a median age of 41.5 months (IQR 11.9 to 74.2) and IFD at a median age of 44.0 months (IQR, 13.7 to 80.1), with a significantly shorter time to IFD compared to children with normal nutritional status (1.73 versus 4.02 months; P=0.007). Children with malnutrition were more likely to die (34.8%

versus 7.5%), compared to children with normal nutritional status (Supplementary Material Table 2.7).

Eleven children were treated for TB during their IFD episodes. The HIV prevalence among children on anti-TB treatment was significantly greater than that of children that were not on TB treatment (63.6% versus 7.0%; P<0.001), and they were significantly more likely to be underweight and stunted (Supplementary Material Table 2.8).

CLWH were significantly older at cancer (93.5 versus 59.2 months; P=0.006) and IFD diagnosis (95.0 versus 64.6 months; P=0.011) than were those without HIV co-infection. Time to IFD was significantly shorter in HIV co-infected children (2.5 versus 4.0 months; P=0.014). Anthropometric measures were significantly lower in CLWH, and high-intensity chemotherapy was utilised in 90% (compared to 49.7% of the children without HIV co-infection; P=0.001) (Supplementary Material Table 2.9).

Timing of first IFD episode

Kaplan-Meier estimates of time to onset of the first IFD episode was significantly shorter in children that were under 18 months of age (1.4 versus 3.8 months), in those with severe malnutrition (1.2 versus 3.8 months), CLWH (1.9 versus 3.5 months), and in children who died as a consequence of the IFD episode (1.2 versus 3.9 months) (Table 2.2 and Supplementary Figure 2.5).

Fungal isolates

Candida albicans (n=71) and *C. parapsilosis* (n=66) were the most commonly isolated species (Figure 2.2). Most of the isolated fungi were identified in IFD episodes, regardless of the underlying cancer diagnosis; however, both IFD episodes in the children with Langerhans cell histiocytosis were due to *C. parapsilosis* (Figure 2.2B). *Candida tropicalis* was isolated in IFD episodes from children with leukaemia and lymphoma only (Figure 2.2).

Table 2.2: Median time to onset of first IFD episode, following cancer diagnosis

Parameters	Number of cases	Median time to first IFD (months; 95% CI)	P-value
Under 18 months at IFD episode			
No	150	3.78 (2.94-4.79)	<0.001
Yes	24	1.4 (0.84-3.27)	
HIV infected			
No	157	3.5 (2.94-4.35)	0.001
Yes	17	1.9 (1.03-3.47)	
Lymphoma			
No	147	3.27 (2.73-4.27)	0.36
Yes	27	2.68 (1.9-6.49)	
Severe malnutrition			
No	147	3.83 (3.06-4.61)	<0.001
Yes	19	1.23 (1-4.23)	
Tuberculosis			
No	155	3.49 (2.96-4.32)	0.51
Yes	10	2.53 (1.03-NA)	
Died			
No	148	3.89 (3.07-4.58)	0.019
Yes	18	1.19 (0.79-2.33)	

Note:
IFD = invasive fungal disease; NA = no estimate. Kaplan-Meier estimates, reporting univariable P-values.

Of the fungal isolates in which fluconazole susceptibility testing was available (n=143), 87.4% were susceptible (including all of the *C. albicans* and *C. tropicalis* isolates) (Table 2.3). Voriconazole susceptibility was 90.9% overall among the 88 isolates that were tested, with all *C. albicans* and *C. tropicalis* isolates being susceptible (Table 2.3).

Susceptibility of isolates to fluconazole and voriconazole were preserved at or above 90% over the study time period for all Candida spp besides *C. krusei* which showed invariable resistance to fluconazole (Figure 2.3).

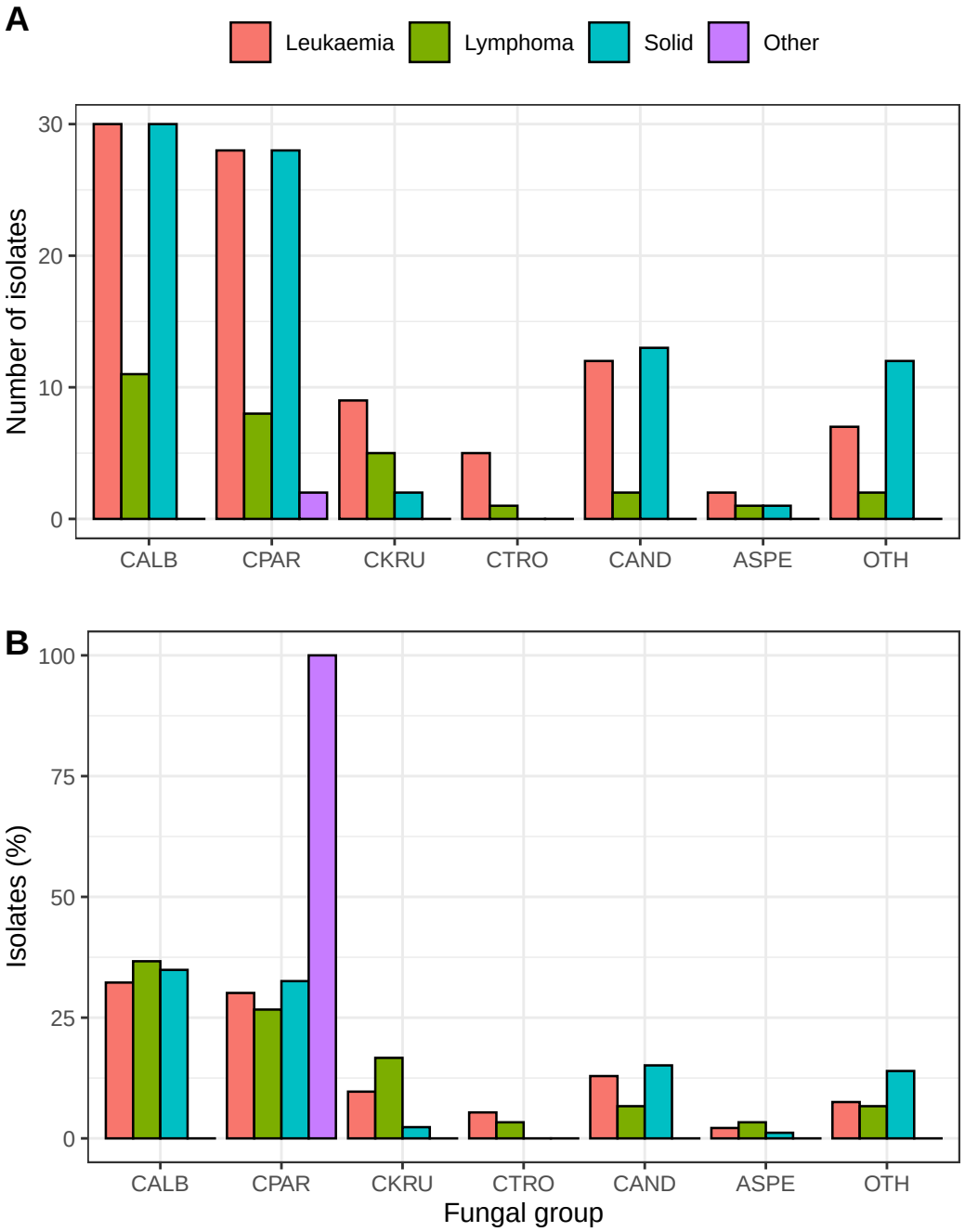

Figure 2.2: Bar chart of fungal isolates stratified by malignancy category

CALB = *Candida albicans*; CPAR = *C. parapsilosis*; CKRU = *C. krusei*; CTRO = *C. tropicalis*; CAND = Candida spp; ASPE = Aspergillus spp; OTH = Other fungal species

Table 2.3: Antifungal susceptibility test results

	Fluconazole			Voriconazole		
Fungal Group	S	I	R	S	I	R
ASPE	NA	NA	NA	100.0% (1)	0.0% (0)	0.0% (0)
CALB	100.0% (57)	0.0% (0)	0.0% (0)	100.0% (10)	0.0% (0)	0.0% (0)
CAND	93.8% (15)	0.0% (0)	6.2% (1)	92.9% (13)	0.0% (0)	7.1% (1)
CKRU	0.0% (0)	0.0% (0)	100.0% (12)	61.5% (8)	23.1% (3)	15.4% (2)
CPAR	90.6% (48)	3.8% (2)	5.7% (3)	95.7% (44)	2.2% (1)	2.2% (1)
CTRO	-	-	-	100.0% (4)	0.0% (0)	0.0% (0)
Total	87.4% (125)	1.4% (2)	11.2% (16)	90.9% (80)	4.5% (4)	4.5% (4)

Note:
ASPE = Aspergillus spp; CALB = Candida albicans; CPAR = C. parapsilosus; CKRU = C. krusei; CTRO = C. tropicalis; CAND = Candida spp; I = intermediate; S = susceptible; R = resistant. One aspergillus isolate underwent susceptibility testing for amphotericin B, and was susceptible (not shown in this table). Itraconazole and micafungin susceptibility tests were not reported by the laboratory.

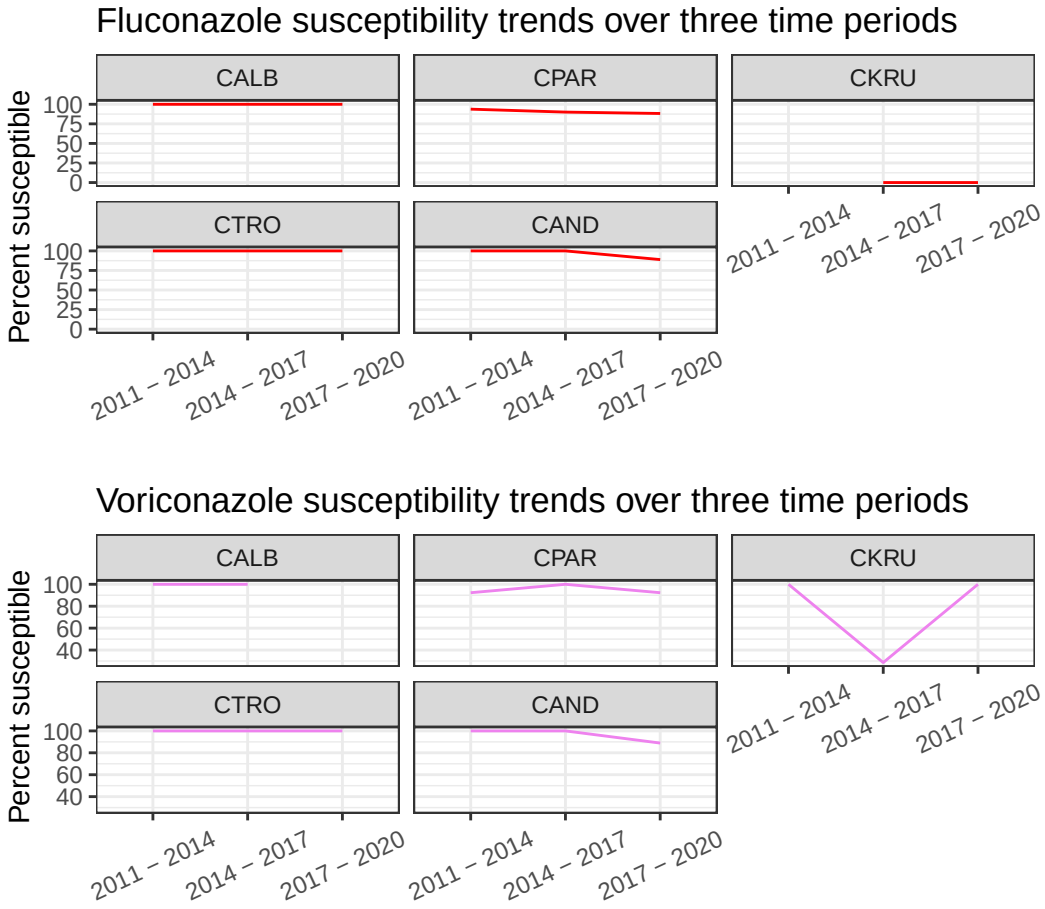

Figure 2.3: Trends in fluconazole and voriconazole susceptibility patterns over time

Biomarkers in IFD diagnosis

CRP was the most frequently used biomarker (n=173 available results). BDG was tested for in 61 IFD episodes, and PCT in 59 IFD episodes. Stratifying the IFD cases between those with severe malnutrition and/or HIV co-infection and/or tuberculosis (designated as a composite risk factor index), no biomarker differed significantly between the groups (Table 2.4).

Table 2.5 shows outputs of the receiver operating characteristic analysis of biomarkers, stratified by children with the composite risk factor for severe disease (those with severe malnutrition and/or HIV co-infection and/or tuberculosis) and those without showed only moderate discrimination between the groups, with a maximum area under the curve of 60.5%, for CRP. Biomarker levels which distinguished between children with and without the composite risk factor were 523.0 pg/mL for BDG, 10.0 mg/L for CRP and 0.15 ug/L for PCT (Table 2.5).

Table 2.4: Median biomarker serum levels, stratified by composite risk factor index

		Composite Risk Factor Index		
	Overall	Not present	Present	P-value
n	196	163	33	
BDG (3 days prior to 7 days post positive fungal culture)	117.00 [31.00, 477.00]	111.50 [31.00, 436.50]	453.00 [18.00, 523.00]	0.426
CRP (3 days prior to 7 days post positive fungal culture)	44.00 [12.00, 126.00]	44.00 [10.75, 115.25]	56.00 [27.00, 161.00]	0.201
PCT (3 days prior to 7 days post positive fungal culture)	0.90 [0.20, 5.00]	0.86 [0.20, 3.84]	1.38 [0.21, 7.56]	0.379
BDG	123.50 [41.75, 432.75]	115.50 [45.25, 346.75]	154.50 [52.00, 517.25]	0.463
CRP	45.00 [12.00, 138.00]	44.00 [11.00, 126.50]	67.00 [28.50, 175.00]	0.068
PCT	0.90 [0.21, 6.75]	0.81 [0.20, 5.14]	1.46 [0.22, 7.58]	0.370

Note:
BDG = beta-D glucan; CRP = C-reactive protein; PCT = procalcitonin. P-values are derived from tests that compare between the IFD cases with the composite risk factor index (i.e. severe malnutrition, and/or HIV infected, and/or tuberculosis diagnosis) and those without the composite risk factor index: Kruskal-Wallis test for comparison of medians. Units of measurement are pg/mL for BDG, mg/L for CRP, and ug/L for PCT.

Table 2.5: Median biomarker serum levels, stratified by composite risk factor index

Biomarker	Cutpoint	Youden Index	Accuracy	Sensitivity	Specificity	AUC
BDG (3 days prior to 7 days post positive fungal culture)	523.00	0.354	0.830	0.444	0.909	0.585
CRP (3 days prior to 7 days post positive fungal culture)	10.00	0.227	0.358	1.000	0.227	0.578
PCT (3 days prior to 7 days post positive fungal culture)	0.15	0.238	0.429	1.000	0.238	0.579
BDG	453.00	0.237	0.744	0.429	0.809	0.562
CRP	10.00	0.225	0.364	1.000	0.225	0.605
PCT	0.15	0.222	0.408	1.000	0.222	0.572

Note:
AUC = area under the curve; BDG = beta-D glucan; CRP = C-reactive protein; PCT = procalcitonin. Receiver operating curves derived from tests that compare between the IFD cases with the composite risk factor index (i.e. severe malnutrition, and/or HIV infected, and/or tuberculosis diagnosis) and those without the composite risk factor index. Units of measurement are pg/mL for BDG, mg/L for CRP, and ug/L for PCT.

Antifungal therapy

Most patients with IFD were treated with fluconazole, amphotericin B or both during their illness episode. The median duration of amphotericin B, fluconazole and micafungin therapy were 9.92 (IQR, 6 to 14), 14 (IQR, 7 to 14) and 13.5 (IQR 10.8 to 16.2) days, respectively (Figure 2.4).

Over the course of the 10-year study period, there was a combined 1549 days of fluconazole use, 1262 days of amphotericin B, 101 days of voriconazole and 27 days of micafungin to treat IFD.

Factors associated with death

Twenty-one (10.7%) of the 197 IFD episodes with known outcome resulted in death (Table 2.1). Lymphoma, severe malnutrition, severe neutropenia, profound neutropenia, prolonged neutropenia, elevated CRP and elevated BDG were associated with death in univariable logistic regression analysis (Supplementary Materials, Table 2.10).

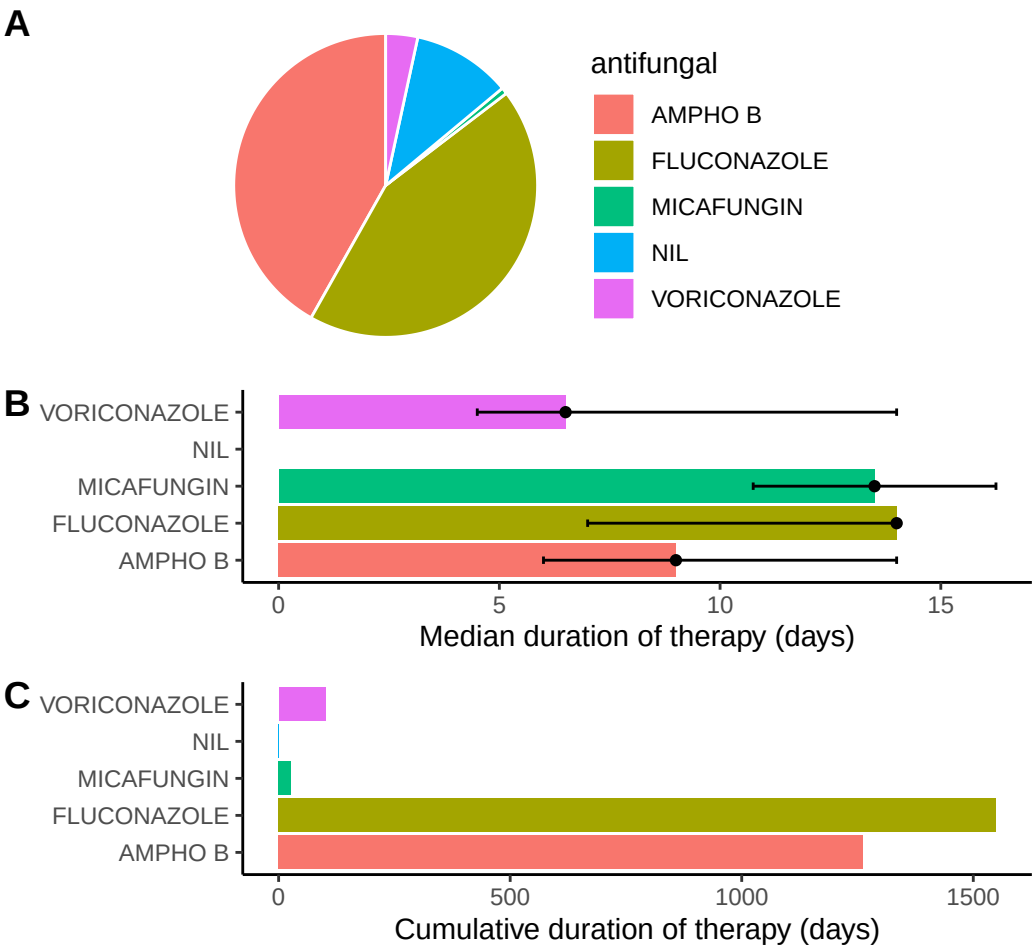

Figure 2.4: Antifungal agents used to treat IFD, mean duration of antifungal therapy, and cumulative duration of therapy

Severe malnutrition and severe neutropenia were independently associated with death in the multivariable model which excluded information on prolonged neutropenia and biomarkers (Final model: n=192 IFD episodes; Table 2.6). These associations were not appreciated when data on prolonged neutropenia and biomarkers were added (Model 2 (n=155 IFD episodes) and Model 3 (n=127 IFD episodes); Supplementary Tables 2.11 and 2.12).

Table 2.6: Final model of factors associated with death in children with with cancer who developed IFD

Parameters	Adjusted Risk Ratio	Lower bound	Upper bound	P-value
Final model, based on 192 IFD episodes with complete data				
Intercept	0.036	0.011	0.100	0.000
Lymphoma	1.746	0.634	4.533	0.263
Age at cancer diagnosis	1.004	0.997	1.011	0.294
Months to IFD	0.932	0.811	1.021	0.229
Severe malnutrition	2.904	1.165	6.918	0.019
Severe neutropenia	4.483	1.835	12.141	0.002

Note:
IFD = invasive fungal disease. Variable selection informed through least absolute shrinkage and selection operator (LASSO) regression.

Discussion

In this study at a paediatric oncology centre in Soweto, South Africa, we demonstrate the serious threat that IFD poses to children with cancer and the consequences thereof. Our findings are important as 80% of children living with cancer reside in LMIC, and there is a paucity of such studies from these countries[12,13].

During the ten-year study period, 174 children and adolescents with cancer had 211 proven IFD episodes. More than half of the episodes occurred in patients with haematological malignancies, and 88% were attributed to Candida spp. Twenty-three episodes occurred in malnourished patients, 11 in patients receiving treatment for TB, and 20 in CLWH. Twenty-one of 197 IFD episodes with known outcome, resulted in death.

The majority of the 211 isolates were Candida spp, with a minority of isolates confirmed Aspergillus spp. This finding of predominantly Candida spp is in keeping with previously published data, however we demonstrated a higher percentage of Candida spp (88.2%) than previously reported (69.0%) by Rosen et al in the United States of America, in 2005[2]. The very small percentage of Aspergillus spp (2%) IFD episodes, in contrast to published studies of up to 18%[2], could likely be accounted for by either the fungal profile in the Unit, or potentially by the relative lack of respiratory samples tested at our institution in children presenting with a febrile illness. Only three out of the 211 isolates were respiratory, whereas

28% of the fungal infection sites in Rosen et al's study were of sinus or respiratory in origin[2]. The possibility that a proportion of IFD in the sinonasal and respiratory systems may be undiagnosed in our setting is concerning. Higher rates of Aspergillus spp have also been described in units with higher rates of haematopoietic stem cell transplantation[26].

Diagnosis of IFD is challenging as the gold standard of diagnosis is tissue histopathology and culture. Tissue biopsies are not always feasible in oncology patients due to the presence of coagulopathies, neutropenia and clinical instability[4]. Additionally, with blood cultures yielding variable rates of positivity (21-71%)[8], it may be difficult to establish a specific diagnosis of IFD. With no adjuvant diagnostic test achieving both good sensitivity and specificity, it may be necessary to combine sets of tests to support the diagnosis of IFD. Our findings showed that PCT has a high level of sensitivity, and could be paired with BDG (highly specific) to potentially give more direction when screening patients at high risk for IFD. More research into these adjunctive tests would be required to make specific recommendations in this regard.

With Candida spp being most prevalent, and fluconazole being the most commonly used antifungal drug in the unit, it is important to note that susceptibility to fluconazole and/or voriconazole was maintained above 90% throughout the study period, except for *C. krusei* which was invariably resistant to fluconazole and had inconsistent sensitivity to voriconazole. With the small proportion of patients presenting with *C. krusei* (7.6%), fluconazole would therefore be the preferred drug to use as potential prophylaxis for the high-risk groups and/or to continue to use as empiric treatment for suspected IFD cases. In our resource-constrained setting, voriconazole use in this regard would not be as feasible due to the cost of the drug. *Candida tropicalis* was isolated in IFD episodes from children with leukaemia and lymphoma only, potentially indicating a predilection for patients treated with more intensive chemotherapeutic regimens.

Risk factors for IFD are well described in the literature, and include type of malignancy, treatment intensity, and therapy-induced complications. The majority of these factors held

true for our patient cohort. Children with IFD were young, with a median age of 65.6 months at diagnosis of their IFD. The majority (58%) of isolates were in patients with haematological malignancies, three-quarters of which were leukaemia. Most of the patients (93%) were receiving moderate or high intensity treatment. These findings are in keeping with previous research describing known risk factors for IFD[19]. This poses a threat to children and adolescents with cancer as leukaemia, specifically acute lymphoblastic leukaemia (ALL), is the most common malignancy in this age group[27], resulting in many patients being at risk for developing IFD.

Unexpectedly, moderate or severe and profound neutropenia preceded the IFD episode in only (48%) of the IFD episodes in our cohort, and 34 (16.1%) IFD episodes were associated with a prolonged, profound neutropenia. The low prevalence of neutropenia in this study could reflect the fact that many of the children in the unit had other comorbidities (such as malnutrition, TB or HIV) that could predispose to IFD in place of a significant neutropenia.

In South Africa, comorbidities of malnutrition, TB and HIV infections are common[13,20,23]. In our study, children and adolescents with severe malnutrition developed IFD sooner after their cancer diagnosis than those that were not severely malnourished. There is a well-established link between malnutrition and infection[15]. Case-fatality rates in children that were severely malnourished was significantly higher (41.2% versus 7.8%) than in those without severe malnutrition. Optimising nutrition should be prioritised in children with cancer.

Children with HIV co-infection were also more likely to develop IFD sooner after their cancer diagnosis, compared to those that were HIV uninfected. Further research into children with cancer and concomitant TB and HIV is warranted to evaluate the relationship to IFD in this population.

Approximately 50% of the IFD episodes manifested within 4 months following cancer diagnosis, and 75% occurred within 8 months. This coincides with the chemotherapy induction phase or shortly thereafter, marked by agents that suppress bone marrow function and immunity, possibly compounded by steroid pulses. Lehrnbecher et al[27] reported an already

compromised immune system in children with ALL at the time of malignancy diagnosis due to reduced oxidative burst activity. Hence, the initial months post-diagnosis and treatment initiation, characterised by immune system challenges, pose a heightened risk of infection, including IFD. This warrants particular attention to monitoring, and potential use of prophylaxis in this period.

The case-fatality rate among children with a known outcome (21/197) was 10.7%, which is lower than mortality rates (20-70%) that have been previously reported[4]. Lehrnbecher[27], however, demonstrated a mortality rate of 12.1% at one year following an IFD in children with ALL, potentially more in keeping with our findings as the majority of our patients had haematological malignancies including ALL. The mortality rate in this context may be lower, as the majority of the isolates were susceptible to the first line treatment in the unit (fluconazole). Furthermore, with the number of immunosuppressive comorbidities in the patients, there is a high index of suspicion for IFD and empiric treatment is often started in ill patients.

In univariable analyses, factors associated with death included lymphoma, severe malnutrition, severe or profound neutropenia and high serum biomarker levels; however, in multivariable analysis only severe malnutrition (aRR 2.90) and severe neutropenia (aRR 4.48) were independently associated with death following an IFD episode, albeit with wide confidence intervals.

Recommendations

Younger children with haematological malignancies on moderate to high intensity chemotherapy regimens and those with comorbidities such as malnutrition, TB or HIV are high-risk for IFD shortly after the diagnosis of a malignancy, and should be prioritised for monitoring and prophylaxis. A high index of suspicion for IFD should be maintained when any oncology patient presents with an illness episode.

The first eight months following a diagnosis of cancer should be recognised as a critical

time for IFD onset and mortality. During this time, active case finding or screening for IFD should be conducted, including sending not only blood and urine, but sinonasal and respiratory specimens for fungal culture if IFD is suspected.

Antifungal prophylaxis during the critical initial period following diagnosis and initiation of chemotherapy may be warranted for more patients than previously recommended. Current guidelines advocate for antifungal prophylaxis in patients with acute myeloid leukaemia (AML), relapsed or high-risk ALL or allogeneic HSCT[28]. More research is required in LMIC to expand this list and cover patients with risk factors such as malnutrition, HIV and TB, which are common.

Our study has limitations. The study was a retrospective, patient folder-based study. The sample size of 211 episodes was relatively small, resulting in estimates with wide confidence intervals. All cases were microbiologically-confirmed, so we could not estimate the burden of total (suspected and confirmed) IFD in the unit, nor could we establish risk factors for IFD because of lack of a control group.

This study is one of the first to describe the characteristics of IFD in children with cancer from a LMIC context, and we aim to bridge the gap in research between our setting and the numerous studies emanating from high-income countries.

Conclusions

IFD is a significant threat to immunocompromised patients, such as children and adolescents with cancer. Younger children with haematological malignancies on moderate to high intensity chemotherapy regimens, and those with comorbidities such as malnutrition, TB or HIV are high-risk for IFD soon after the diagnosis of a malignancy, and should be prioritised for antifungal prophylaxis. Severe malnutrition and severe neutropenia were independently associated with death. The presence of these risk factors should prompt clinicians to pre-empt IFD in the face of febrile neutropenia in children with cancer in settings such as ours.

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Supplementary materials

Table 2.7: Clinical characteristics of IFD episodes, stratified by nutritional status

	Normal nutrition	Severe malnutrition	P-value
n	174	23	
Male (%)	83 (47.7)	17 (73.9)	0.032
Age (months) at cancer Dx [IQR]	62.09 [26.92, 103.66]	41.52 [11.90, 74.21]	0.141
Age (months) at IFD [IQR]	66.33 [30.24, 109.36]	44.00 [13.72, 80.08]	0.094
Time from Dx to IFD onset (months) [IQR]	4.02 [1.99, 7.70]	1.73 [0.90, 4.15]	0.007
HIV Positive (%)	16 (9.2)	4 (17.4)	0.392
Mean WFA Z-Score (SD)	-0.66 (1.38)	-2.52 (1.36)	-
Median HFA Z-Score [IQR]	-0.92 [-1.96, -0.02]	-1.04 [-2.24, -0.26]	-
Mean WFH Z-Score (SD)	-0.51 (1.36)	-3.47 (1.04)	-
Median BMI Z-Score [IQR]	-0.54 [-1.48, 0.48]	-3.55 [-3.96, -2.81]	-
Treatment intensity			0.176
Low	12 (6.9)	3 (13.0)	
Moderate	67 (38.5)	12 (52.2)	
High	95 (54.6)	8 (34.8)	
ANC Category (%)			0.045
Normal or mild	94 (55.3)	6 (27.3)	
Moderate or severe	31 (18.2)	6 (27.3)	
Profound	45 (26.5)	10 (45.5)	
Prolonged profound ANC (%)	24 (17.3)	6 (37.5)	0.108
Fungal group (%)			0.129
Candida albicans	59 (33.9)	8 (34.8)	
Candida parapsilosis	60 (34.5)	5 (21.7)	
Candida krusei	11 (6.3)	1 (4.3)	
Candida tropicalis	4 (2.3)	2 (8.7)	
Other Candida spp	17 (9.8)	6 (26.1)	
Aspergillus spp	4 (2.3)	0 (0.0)	
Other fungi	19 (10.9)	1 (4.3)	
Died (%)	13 (7.5)	8 (34.8)	<0.001

Note:
HIV = human immunodeficiency virus type 1; HFA = height-for-age; IFD = invasive fungal disease; IQR = interquartile range; WFA = weight-for-age; WFH = weight-for-height. P-values are derived from tests that compare across nutritional status: Chi-square test or Fisher's exact test, as appropriate, for categorical variables; Student t test for comparison of means; Kruskal-Wallis test for comparison of medians.

Table 2.8: Clinical characteristics of IFD episodes, stratified by tuberculosis status

	Not tuberculosis	Tuberculosis	P-value
n	185	11	
Male (%)	91 (49.2)	9 (81.8)	0.073
Age (months) at cancer Dx [IQR]	59.58 [23.42, 96.82]	71.39 [44.62, 168.60]	0.161
Age (months) at IFD [IQR]	65.43 [29.04, 105.35]	78.39 [50.45, 175.92]	0.162
Time from Dx to IFD onset (months) [IQR]	3.94 [1.90, 7.00]	2.68 [1.11, 9.65]	0.722
HIV Positive (%)	13 (7.0)	7 (63.6)	<0.001
Mean WFA Z-Score (SD)	-0.82 (1.49)	-2.14 (1.33)	0.034
Median HFA Z-Score [IQR]	-0.86 [-1.94, 0.04]	-2.61 [-3.77, -1.37]	0.001
Mean WFH Z-Score (SD)	-0.85 (1.65)	-1.16 (1.27)	0.540
Median BMI Z-Score [IQR]	-0.73 [-1.89, 0.40]	-0.99 [-1.77, -0.31]	0.590
Treatment intensity			0.069
Low	13 (7.0)	2 (18.2)	
Moderate	77 (41.6)	1 (9.1)	
High	95 (51.4)	8 (72.7)	
ANC Category (%)			0.433
Normal or mild	95 (52.8)	4 (36.4)	
Moderate or severe	35 (19.4)	2 (18.2)	
Profound	50 (27.8)	5 (45.5)	
Prolonged profound ANC (%)	27 (18.6)	3 (33.3)	0.517
Fungal group (%)			0.281
Candida albicans	60 (32.4)	7 (63.6)	
Candida parapsilosis	63 (34.1)	2 (18.2)	
Candida krusei	12 (6.5)	0 (0.0)	
Candida tropicalis	5 (2.7)	1 (9.1)	
Other Candida spp	23 (12.4)	0 (0.0)	
Aspergillus spp	4 (2.2)	0 (0.0)	
Other fungi	18 (9.7)	1 (9.1)	
Died (%)	20 (10.8)	1 (9.1)	1.000

Note:
HIV = human immunodeficiency virus type 1; HFA = height-for-age; IFD = invasive fungal disease; IQR = interquartile range; WFA = weight-for-age; WFH = weight-for-height. P-values are derived from tests that compare across tuberculosis status: Chi-square test or Fisher's exact test, as appropriate, for categorical variables; Student t test for comparison of means; Kruskal-Wallis test for comparison of medians.

Table 2.9: Clinical characteristics of IFD episodes, stratified by HIV status

	HIV-uninfected	HIV-infected	P-value
n	191	20	
Male (%)	93 (48.7)	13 (65.0)	0.249
Age (months) at cancer Dx [IQR]	59.23 [23.34, 96.32]	93.50 [53.30, 173.44]	0.006
Age (months) at IFD [IQR]	64.60 [28.88, 104.55]	94.98 [62.79, 175.36]	0.011
Time from Dx to IFD onset (months) [IQR]	4.00 [1.86, 7.76]	2.52 [0.97, 3.69]	0.014
Tuberculosis (%)	4 (2.3)	7 (35.0)	<0.001
Mean WFA Z-Score (SD)	-0.74 (1.45)	-2.42 (1.08)	<0.001
Median HFA Z-Score [IQR]	-0.82 [-1.81, 0.11]	-2.16 [-3.24, -1.90]	<0.001
Mean WFH Z-Score (SD)	-0.78 (1.65)	-1.57 (1.33)	0.039
Median BMI Z-Score [IQR]	-0.65 [-1.88, 0.43]	-1.14 [-2.01, -0.47]	0.084
Treatment intensity			0.001
Low	13 (6.8)	2 (10.0)	
Moderate	83 (43.5)	0 (0.0)	
High	95 (49.7)	18 (90.0)	
ANC Category (%)			0.522
Normal or mild	99 (53.2)	8 (40.0)	
Moderate or severe	34 (18.3)	5 (25.0)	
Profound	53 (28.5)	7 (35.0)	
Prolonged profound ANC (%)	31 (20.4)	3 (20.0)	1.000
Fungal group (%)			0.114
Candida albicans	59 (30.9)	12 (60.0)	
Candida parapsilosis	60 (31.4)	6 (30.0)	
Candida krusei	16 (8.4)	0 (0.0)	
Candida tropicalis	5 (2.6)	1 (5.0)	
Other Candida spp	26 (13.6)	1 (5.0)	
Aspergillus spp	4 (2.1)	0 (0.0)	
Other fungi	21 (11.0)	0 (0.0)	
Died (%)	17 (9.6)	4 (20.0)	0.296

Note:
HIV = human immunodeficiency virus type 1; HFA = height-for-age; IFD = invasive fungal disease; IQR = interquartile range; WFA = weight-for-age; WFH = weight-for-height. P-values are derived from tests that compare across HIV status: Chi-square test or Fisher's exact test, as appropriate, for categorical variables; Student t test for comparison of means; Kruskal-Wallis test for comparison of medians.

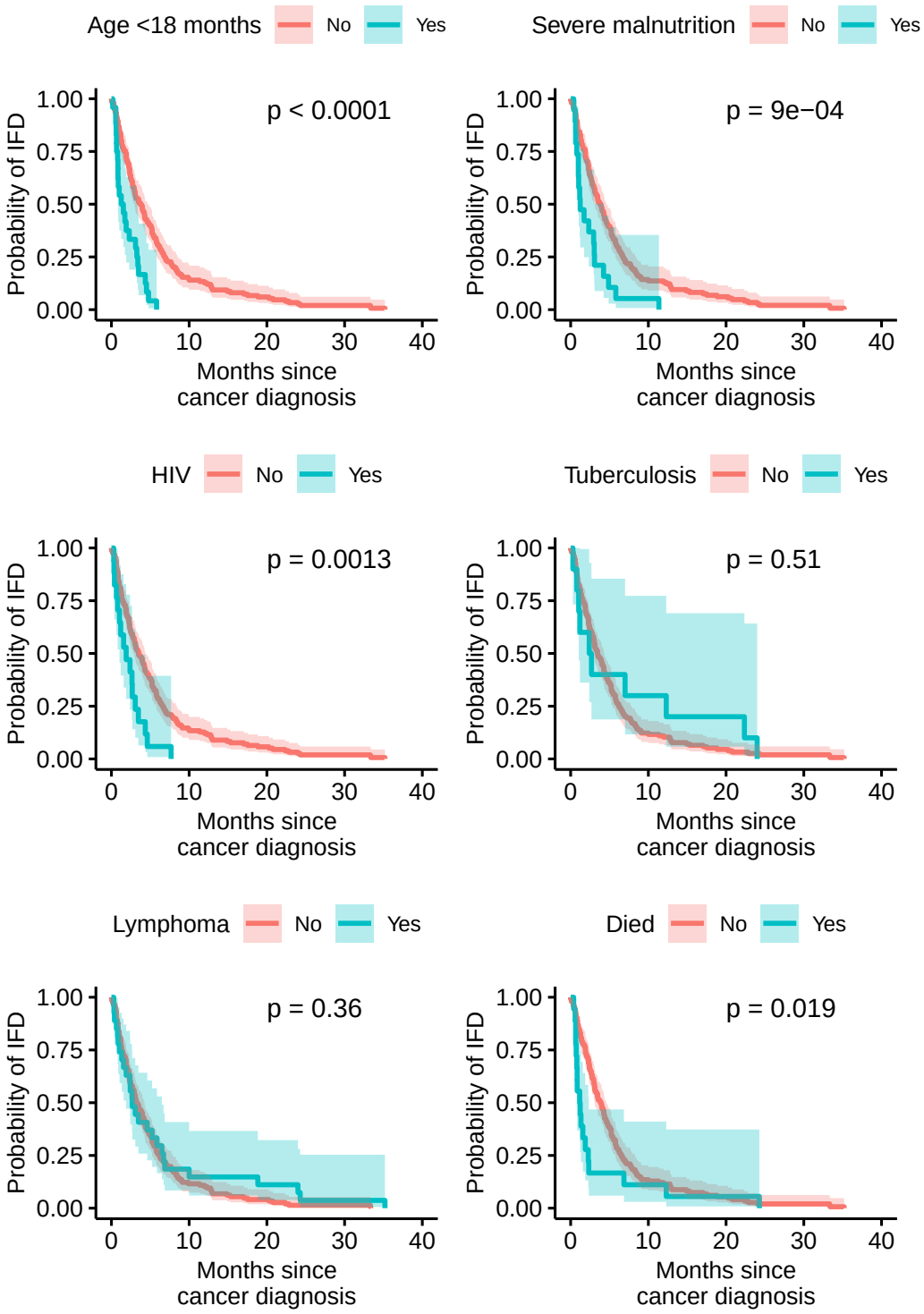

Figure 2.5: Survival curves for time to onset of first IFD epidose, stratified by key clinical characteristics

Table 2.10: Univariable analysis of factors associated with death as the outcome of interest

Variable	Relative Risk	Lower	Upper	P-value
Male	1.576	0.683	3.639	0.288
Tumour type				
Lymphoma	2.656	1.014	6.959	0.048
Solid tumours	0.777	0.285	2.124	0.624
Other	0.000	0.000	Inf	0.992
Lymphoma compared to other cancers	3.018	1.275	7.146	0.013
Age at Dx	1.005	0.999	1.012	0.108
Age at IFD	1.005	0.998	1.011	0.165
Months to IFD	0.878	0.748	1.030	0.112
Anthropometry				
Wt-for-age Z-score	0.754	0.494	1.151	0.192
Height-for-age Z-score	1.049	0.871	1.264	0.613
Weight-for-height Z-score	0.655	0.514	0.833	0.001
BMI-for-age Z-score	0.657	0.512	0.843	0.001
Co-morbidities				
Severe malnutrition	4.656	2.016	10.749	<0.001
HIV infected	2.082	0.740	5.861	0.166
Tuberculosis	0.841	0.125	5.665	0.859
Chemotherapy				
Medium Intensity Rx	1.139	0.152	8.557	0.899
High Intensity Rx	2.039	0.295	14.080	0.471
Degree of neutropenia				
Moderate neutropenia	0.541	0.070	4.167	0.556
Severe neutropenia	5.455	2.083	14.285	0.001
Profound neutropenia	6.227	2.536	15.289	<0.001
Prolonged neutropenia	5.093	2.226	11.650	<0.001
Fungal species				
Candida spp	0.439	0.065	2.983	0.401
Other fungal spp	0.200	0.014	2.803	0.234
Biomarkers				
CRP	1.005	1.003	1.008	<0.001
PCT	1.007	0.986	1.029	0.503
BDG	1.006	1.001	1.012	0.033
Composite biomarker index	1.909	0.833	4.375	0.128

Note:
BDG = beta-D-glucan; CRP = C-reactive protein; Dx = diagnosis; IFD = invasive fungal disease; HIV = human immunodeficiency virus; RR = risk ratio; Rx = treatment. Composite biomarker index was assigned to patients with either BDG and CRP, or CRP and PCT, or BDG and PCT levels above the cut-offs (i.e. 523 pg/mL for BDG, 10 mg/L for CRP, 0.15 ug/L for PCT) identified in the receiver operator characteristic analysis presented in Table 2.4.

Table 2.11: LASSO Regression coefficients for parameters to include in the final Poisson regression models

Parameters	Final Model	Model 2	Model 3
Age at cancer diagnosis	0.000	0.001	0.000
Age at IFD	0.000	0.000	0.000
Composite biomarker index			0.000
HIV status	0.000	0	0.000
Intercept	-0.246	-0.303	-0.288
Lymphoma	0.094	0.068	0.085
Months to IFD	-0.003	-0.003	-0.004
Prolonged neutropenia		0.189	0.195
Severe malnutrition	0.213	0.249	0.251
Severe neutropenia	0.172	0.059	0.050

Note:
LASSO = least absolute shrinkage and selection operator. Composite biomarker index was assigned to patients with either BDG and CRP, or CRP and PCT, or BDG and PCT levels above the cut-offs (i.e. 523 pg/mL for BDG, 10 mg/L for CRP, 0.15 ug/L for PCT) identified in the receiver operator characteristic analysis presented in Table 2.4.

Table 2.12: Candidate models of factors associated with death in children with with cancer who developed IFD

Parameters	Adjusted Risk Ratio	Lower bound	Upper bound	P-value
Model 2, based on 155 IFD episodes with complete data				
Intercept	0.038	0.011	0.112	0.000
Lymphoma	1.381	0.474	3.720	0.535
Age at cancer diagnosis	1.005	0.998	1.012	0.178
Months to IFD	0.948	0.832	1.034	0.326
Severe malnutrition	2.533	0.951	6.342	0.054
Severe neutropenia	2.501	0.664	8.872	0.155
Prolonged neutropenia	2.194	0.774	7.411	0.165
Model 3, based on 127 IFD episodes with complete data				
Intercept	0.060	0.014	0.207	0.000
Lymphoma	1.549	0.474	4.774	0.452
Age at cancer diagnosis	1.003	0.995	1.011	0.399
Months to IFD	0.922	0.771	1.029	0.265
Severe malnutrition	2.328	0.807	6.370	0.106
Severe neutropenia	1.992	0.476	7.769	0.319
Prolonged neutropenia	1.977	0.622	7.545	0.274
Composite biomarker index	1.148	0.433	2.901	0.774

Note:
IFD = invasive fungal disease. Composite biomarker index was assigned topatients with either BDG and CRP, or CRP and PCT, or BDG and PCTlevels above the cut-offs (i.e. 523 pg/mL for BDG, 10 mg/L for CRP, 0.15 ug/L for PCT) identified in the receiver operator characteristic analysispresented in Table 2.4. Variable selection informed through least absolute shrinkage and selection operator (LASSO) regression.

APPENDIX 2: Ethics Approval Certificates

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R49 Dr A Keene

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M210701

NAME: (Principal Investigator)	Dr A Keene
DEPARTMENT:	School of Clinical Medicine Department of Paediatrics and Child Health Medical School University
PROJECT TITLE:	*Invasive fungal disease in children with cancer: a ten year analysis from a paediatric oncology centre in Soweto, South Africa*
DATE CONSIDERED:	2021/07/30
DECISION:	Approved unconditionally
CONDITIONS:	
NOTE:	If contact information regarding student study participants is required, please contact the Registrar's office - <Nicoleen.Potgieter@wits.ac.za>
SUPERVISOR:	Professors G Naidu & D Moore; Dr J Wadula
APPROVED BY:	CB Penny Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	2021/10/04

This Clearance Certificate is valid for 5 years from the date of approval. An extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office secretariat on the 3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to submit details to the Committee. **I agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in July and therefore reports and re-certification will be due in the month of **July** each year. Unreported changes to the study may invalidate the clearance given by the HREC (Medical).

Signature of Principal Investigator _____ Date _____

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11 November 2021

Applicant: Abigail Keene
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CC: Jeanette Wadula – Medical Microbiology

Re: Approval to access National Health Laboratory Service (NHLS) Data

Your application to undertake a research project **"Invasive Fungal Disease in Children with Cancer: a ten-year analysis from a Paediatric Oncology Centre in Soweto, South Africa" Ref No: PR2119246** using data from the NHLS database has been reviewed. This letter serves to advise that the application has been approved and the required data will be made available to you ***without patient names*** to conduct the proposed study as outlined in the submitted application. Submissions should be made annually on the AARMS system – https://aarms.nhls.ac.za.

Please note that approval is granted on your compliance with the NHLS conditions of service and that the study can only be undertaken provided that the following conditions have been met.

- Processes are discussed with the relevant NHLS departments (i.e. Information Management Unit and Operations Office) and are agreed upon.
- Confidentiality is maintained at participant and institutional level and there is no disclosure of personal information or confidential information as described by the NHLS policy.
- NHLS Data cannot be used to track patients as no pre-approval/consent is obtained from Patients.
- All data requested should be in accordance with the research protocol submitted and approved by the relevant Ethics Committee.
- Request for the inclusion of the NHLS as a source of data in the original protocol to be approved by Ethics as NHLS does not have a Human Research Ethics Committee.
- A final report of the research study and any published paper resulting from this study are submitted and addressed to the NHLS Academic Affairs and Research office and the NHLS has been acknowledged appropriately.
- Jeanette Wadula is noted as NHLS collaborator for this study.

Please note that this letter constitutes approval by the NHLS Academic Affairs and Research Office. Any data related queries may be directed to NHLS Corporate Data Warehouse, contact number: 011 386 6074 email: zarina.sabat@nhls.ac.za

P.P Malope-Kgokong

Dr Babatyi Malope-Kgokong
National Manager: Academic Affairs and Research

APPENDIX 3: Study Protocol

Invasive Fungal Disease in Children with Cancer: a ten-year analysis from a Paediatric Oncology Centre in South Africa

Research Protocol 2021

Researcher: Abigail Keene
MBBCh (Wits)

Student Number: 362950

W-23-01-102

Degree: Master of Medicine in the Discipline of Paediatrics – MMed (Paed)

Supervisor: Professor Gita Naidu
MBChB, FCPaed (SA), MMed (Paed), PhD
Head of Department: Paediatric Haematology Oncology at Chris Hani Baragwanath Hospital, South Africa

Supervisor: Professor David Paul Moore
MBBCh, FCPaed (SA), MMed (Paed), Cert (Paed) ID, MPhil (Paed ID), PhD
Paediatric Infectious Diseases Specialist, Chris Hani Baragwanath Academic Hospital and University of the Witwatersrand, South Africa

Supervisor: Dr Jeannette Wadula
MBBCh, FC Path(SA)Micro, DTM&H, Dip in Med
Head of Department: Microbiology at the Chris Hani Baragwanath Academic Hospital National Health Laboratory Service, South Africa

Contents

Nomenclature

ALL	acute lymphoblastic leukaemia
AML	acute myeloid leukaemia
ANC	absolute neutrophil count
ART	antiretroviral treatment
BDG	β-D-glucan
CHBAH	Chris Hani Baragwanath Academic Hospital
CRP	C-reactive protein
HIV	human immunodeficiency virus type-1
IFD	invasive fungal disease
GM	galactomannan
HSCT	haematopoietic stem cell transplantation
LMIC	low- and middle-income countries
MAM	moderate acute malnutrition
MUAC	mid-upper arm circumference
NHLS	National Health Laboratory Service
PCR	polymerase chain reaction
PCT	procalcitonin
TB	tuberculosis
TPN	total parenteral nutrition
SAM	severe acute malnutrition
WHO	World Health Organization

1. Introduction

1.1 Background and rationale for study

Invasive fungal diseases (IFD) are opportunistic infections, rare in patients with a normally functioning immune system[1]. However, they pose a significant threat to immunocompromised patients such as children with cancer[2], and in this population the most common cause of treatment-related morbidity and mortality is infections[3]. There has been a trend of increased survival in paediatric oncology patients over recent years owing to the improvement in cancer treatment regimens as well as improved antibiotic therapies[1, 3]. The advancements in chemotherapy, surgical techniques, radiotherapy and bone marrow transplantation predispose these patients to longer and more severe episodes of immunosuppression with periods of profound, prolonged neutropenia. This places them at an increased risk for infections, including IFD, and increases exposure to broad-spectrum antibiotics, which also predispose to IFD[1-3]. While IFD causes significant morbidity and mortality[2], there is a paucity of studies published on the topic in low- and middle-income countries (LMIC), including South Africa. Mortality from IFD varies from 20-70% depending on the degree of immunosuppression, underlying comorbidities, the severity of the infection as well as the time to diagnosis and initiation of appropriate antifungal therapy[4]. The ability to diagnose and treat IFD is a vital component in successfully treating children with cancer.

1.2 Fungal Diseases

Fungal yeasts and moulds cause a variety of clinical presentations as a result of the interaction between the fungal pathogen and their human host. The host immune response, underlying malignant disease and its treatment, as well as comorbidities impact on the presentation and severity of IFD in children with cancer[3,5]. Signs and symptoms of IFD may be absent until such time as there is immune reconstitution in severely immunocompromised hosts[5]. It is therefore imperative to have a strong clinical suspicion of IFD, and to actively look for evidence in any high-risk patient, especially with a persistent fever despite prolonged broad-spectrum antibiotic use (more than 96 hours) and expected prolonged neutropenia (of at least ten days) [6].

Rosen et al described IFD as the isolation of a fungus from a bodily fluid or tissue that is normally sterile, with accompanying constitutional symptoms (for example, fever)[2]. There can also be pathologic or radiographic evidence of invasive infection despite no growth on cultures of clinical specimens[2]. IFD are classified as proven, possible or probable based on the certainty of the diagnosis[1].

1.2.1 Proven IFD

Criteria for proven mould infection include the histopathological, cytopathological or microscopic finding of hyphae in association with tissue damage on examination of a sterile tissue specimen, or a blood culture that grows a mould[1]. A diagnosis of proven yeast infection is established by histopathological, cytopathological or microscopic findings of yeast cells on a sterile specimen, or by demonstrating yeast on a culture of a sterile site with clinical or radiological findings consistent with infection[1]. Proven *Cryptococcus neoformans* infection is also demonstrated by the presence of cryptococcal antigen in a patient's blood or cerebrospinal fluid[1].

1.2.2 Possible or probable IFD

Possible or probable fungal infection depends on specific host factors, with the presence of clinical criteria consistent with IFD as well as mycological evidence of infection, but where blood cultures remain negative for fungal pathogens[1]. Host factors include prolonged, profound neutropenia, T-cell immunosuppressive therapy or an inherited immunodeficiency as well as the use of corticosteroids, or a haematopoietic stem cell transplant and the frequent and prolonged use of broad-spectrum antibiotics. One of these must be present with clinical criteria suggestive of IFD: radiographic features suggestive of a fungal infection of the lower respiratory tract, sinuses or central nervous system or evidence of tracheobronchitis on bronchoscopy. There should also be evidence of mould in respiratory tract samples – sputum or bronchoalveolar lavage, or positive non-culture or indirect tests such as galactomannan (GM) or β-D-glucan (BDG)[1].

1.3 Fungal species implicated in IFD

The most common fungal pathogens in paediatric oncology patients are *Candida* species and *Aspergillus* species[1, 2].

1.3.1 Yeast – *Candida* species

Candida species are responsible for the majority of IFD in paediatric oncology patients[4], causing up to 69% of IFD in this population in a study by Rosen et al in 2005[2]. *Candida* species are part of the normal gastrointestinal flora and, while they can cause localized or superficial infections of the immunocompetent host's mucous membranes, they can cause disseminated disease in the immunocompromised patient[3]. Yeast cells enter the bloodstream at points of mucosal or cutaneous breakdown such as at central venous catheter insertion sites or chemotherapy-induced mucosal disruption[1,3]. The most common presentation is fever that is refractory to broad-spectrum antibiotics[4]. Severe sepsis and shock occurs in about 30% of patients[4].

Up to half of all patients with proven invasive candidiasis will have a negative blood culture due to the low sensitivity of the blood cultures for fungi[5]. Automated blood culture systems, such as the BacT/Alert, which is available in our institution, have a sensitivity of about 50% for invasive candidiasis[4]. Candidaemia occurs when there is a positive blood culture in the absence of deep-seated infection. Candida infections can disseminate to various sites including the central nervous system, eyes, liver, spleen, lungs and kidneys. Disseminated or deep-seated fungal infection (typically hepatosplenic) can also occur in the absence of candidaemia or a positive culture[7].

1.3.2 Moulds – *Aspergillus* species

Invasive aspergillosis has a predilection for both the upper and lower respiratory tracts. In primary invasive aspergillosis of the lungs, about half of patients have signs and symptoms of a respiratory tract infection, while up to 62% of patients with sinonasal disease can remain asymptomatic[5]. It is therefore likely that invasive aspergillosis is under-diagnosed and undertreated in the paediatric

population. Aspergillosis can also present as a skin lesion, which is more common in children than in adults. Cutaneous aspergillosis can occur as a result of direct spore inoculation, or from haematogenous spread as part of disseminated disease[1,5].

1.4 Fungal disease diagnosis

The gold standard in diagnosis of IFD is tissue histopathology and culture[8]. Tissue biopsies are not always feasible in oncology patients due to the presence of coagulopathies, neutropenia and clinical instability[4]. Blood cultures are only capable of detecting fungal cells that are still viable. A negative blood culture may therefore demonstrate either a lack of viable fungal cells in the bloodstream, an insufficient concentration of viable cells, intermittent release of viable fungal cells into the circulation, treatment with antifungal therapy prior to specimen collection, or suboptimal sample volume[7]. The exact sensitivity of fungal blood cultures ranges with different studies, but Clancy et al[7] described that in autopsy-proven invasive candidiasis in adults, the sensitivity of blood cultures drawn prior to the patients' demise was 21-71%[7]. Fungal cultures also have a median time to positivity of two to three days, which can further delay appropriate treatment and worsen morbidity and mortality[7].

It is therefore important to review adjunctive methods of screening for and diagnosing IFD. These include 'non-culture' biomarkers that are fungal or pathogen-derived, such as BDG, GM (specifically for *Aspergillus* species) and polymerase chain reaction (PCR) identifying fungal DNA[8]. These 'non-culture' tests seem to exhibit high negative predictive values (up to 97%) with low positive predictive values (8-23%), which limits their use in diagnosing IFD. More studies need to be done on these adjunctive diagnostic methods in paediatrics[1], however current recommendations by the International Paediatric Febrile Neutropenia Guidelines do not advocate their use in diagnosing IFD in children[1, 9].

1.5 Risk factors for IFD

There are established risk factors for the high incidence of IFD in children with cancer, including the underlying cancer diagnosis and therapy-induced complications[5,6,10]. High-risk malignancies and treatments include acute myeloid leukaemia (AML), high risk acute lymphoblastic leukaemia (ALL) including relapsed disease, highly myelosuppressive chemotherapy, and allogeneic haematopoietic stem cell transplantation (HSCT)[6]. Therapy-induced complications include the presence of neutropenia: profound with an absolute neutrophil count (ANC) of less than 500 cell/µl and prolonged (lasting at least seven to ten days)[5,10]. Other recognised risk factors include the use of total parenteral nutrition (TPN), chemotherapy agents and other immunosuppressive drugs used during treatment such as high dose corticosteroids[5]. Corticosteroids at an equivalent dose of prednisone of 2 mg/kg/day are considered to confer high risk for the development of IFD[4]. This is owing to steroid-induced impairment of phagocytosis, neutrophil function and the humoral immune response[4]. Chemotherapy-induced mucosal injury, and intravenous, central venous and indwelling catheters are recognized risk factors for IFD[5,6,10]. Concurrent or preceding bacterial infection in these immunocompromised patients requiring the use of broad-spectrum antibiotics also predisposes to IFD[5].

In the South African setting, we have the added burden of other comorbidities common in our population, including malnutrition, human immunodeficiency virus type-1 (HIV) and tuberculosis (TB).

1.5.1 Malnutrition

Eighty per cent of children with cancer live in LMIC[11,12]. Malnutrition is endemic in LMICs with a prevalence of up to 50-70% recorded[12]. In a study in Pretoria in 2008, 21.6% of paediatric oncology patients were wasted at the time of their diagnosis while a further 24.3% were underweight[13]. Malnutrition has significant consequences in the treatment and survival of malignancies. There is a well-established connection between malnutrition and increased risk of

infection[14] as it is associated with depressed cell-mediated immunity[15], decreased antibody affinity[15] and higher frequency and longer duration of neutropenia in oncology patients[13,15] as well as more severe neutropenia (lower nadir of the ANC)[11,14]. Malnutrition has also been associated with higher chemotherapy-associated toxicity rates[12] and reduced tolerance to therapy resulting in treatment delays, longer hospital stays and occasionally abandonment of care[13] with increased morbidity and mortality in these patients[11].

Malnutrition in children with cancer is multifactorial and the factors associated include the socioeconomic status of the child, the educational status of the mother[16], the type of cancer and the treatment of the cancer[17]. Cancer and cancer therapy induces a catabolic state, which leads to a reduction in lean body mass and malnutrition. Treatment-associated factors such as nausea and vomiting, oral mucositis, poor appetite and food aversions play a significant role in reduced nutrient intake and subsequent malnutrition[17].

Malnutrition is therefore an important comorbidity to identify as it has significantly negative consequences on survival rates, despite being treatable. It is specifically important with regards to IFD, as it causes a statistically significant increase in the duration and frequency of neutropenia, which is the most significant and commonly identified risk factor for IFD[18]. A study conducted in Malawi showed that patients with Burkitt Lymphoma who had profound and prolonged neutropenia during their treatment, as well as patients with treatment-related deaths, had malnutrition at the time of their oncological diagnosis[14].

1.5.2 HIV

In 2018, there were 260,000 children with HIV living in South Africa, of which about 63% were on antiretroviral treatment (ART)[19]. The complications of HIV have been well described, including an increased predisposition for opportunistic infections, including IFD, as a consequence of HIV-induced immunodeficiency and immune dysfunction[11]. HIV is therefore an independent risk factor for the development of IFD.

1.5.3 Tuberculosis

South Africa has a high incidence of TB with 301 per 100,000 population in 2018[20]. Children with cancer are immunocompromised owing to the disease itself as well as the treatment they receive and are therefore at a higher risk of *Mycobacterium tuberculosis* infection progressing to disease. A study published in Johannesburg in 2020 looked at 169 children with various malignancies and screened them for TB. Five children (2.9%) were diagnosed with TB prior to treatment of the cancer, and a further 34 (20.7%) were diagnosed and treated following initiation of cancer treatment[20]. Furthermore, after the diagnosis and treatment of TB, these children had a higher number of episodes of sepsis with prolonged neutropaenia, compared with before the development of TB[20]. This may put them at a higher risk for developing IFD.

1.6 Treatment of IFD

The ideal treatment of IFD should include not only appropriate antifungal agents, but also surgical resection where feasible and reconstitution of the immune system[1]. While appropriate antifungal drugs are the mainstay of treatment, they can be inadequate if used alone, as there is poor penetration of the drugs into areas of tissue necrosis caused by invasive infection. Therefore, when surgical resection is combined with antifungal agents, the odds of infection resolution are greater[1]. Immune reconstitution is key in the treatment of paediatric oncology patients with IFD[1]. Therefore, immunosuppressive medications including chemotherapy and corticosteroids should be minimized if possible until resolution of the IFD. The use of granulocyte colony-stimulating factor to promote neutrophil recovery can be used as an adjunct in treating IFD in patients with low neutrophil counts[1].

1.6.1 Antifungal drugs

There are three classes of antifungal agents commonly used in children with cancer and fungal infection: polyenes, triazoles and echinocandins[3].

Polyenes include amphotericin B, an antifungal with a broad spectrum of activity, effective against yeasts and moulds. It has good penetration into most tissues, is widely available and affordable, and is therefore the standard antifungal drug in most LMIC[3]. Amphotericin B however, is an intravenous-only medication and has recognised side effects such as renal toxicity and dysfunction, electrolyte imbalances (such as hypokalaemia) and acute allergic reactions (which may manifest as fever, chills, hypotension, nausea and vomiting)[3].

Triazoles include fluconazole and voriconazole. Fluconazole is commonly used in children with cancer for prophylaxis against IFD. It is used in mucosal infections as well as a step-down or continuation therapy for invasive candidiasis following initial therapy with a polyene or echinocandin[3]. Fluconazole is relatively inexpensive and is widely available in LMICs, however it does not have suitable activity against moulds such as *Aspergillus* species, and has drug interactions with many other medications commonly used in our setting – rifampicin, antiepileptic drugs, protein pump inhibitors and macrolides all affect the drug levels of fluconazole[3].

Echinocandins include micafungin. This drug disrupts the fungal cell wall and is fungicidal against *Candida* species and fungistatic against *Aspergillus* species[3]. It is therefore recommended for first line use in invasive *Candida* species infections and as a second line or adjunct therapy for aspergillosis. The drug has no activity against fungi such as *Cryptococcus neoformans* and has poor central nervous system penetration[3].

1.7 Rationale for the Study

IFD is a serious, significant issue in paediatric oncology with few studies specifically dedicated to the topic. Risk factors, diagnostic methods and treatment in higher income settings are described in the literature. However, in our LMIC setting we have many additional risk factors for the development of IFD, as well as barriers to diagnostics and treatment. Paediatric patients are as susceptible to IFD as adult patients, however the two groups are significantly different with regards to their physiology, comorbidities and their tolerance to treatment and therefore extrapolation of

principles from adult studies are not always appropriate[4]. In order not to fail our patients, we must look at how we can better prevent, diagnose, monitor and treat IFD. Further research is thus pertinent to add insight into IFD in paediatric cancer patients in LMIC.

1.8 Study Aim

To describe the epidemiology of invasive fungal disease in children with cancer, diagnostic parameters, antifungal treatment and patient outcomes in our LMIC setting.

1.8.1 Objectives

- To describe the prevalence of IFD in children with cancer, treated at the Chris Hani Baragwanath Academic Hospital (CHBAH) Paediatric oncology unit
- To describe the most commonly cultured fungal species in the unit
- To assess the antifungal susceptibility profiles of the fungi cultured, and to establish whether the antifungal susceptibility profiles have changed over time
- To describe the types of malignancy most commonly associated with IFD in this setting
- To evaluate the ANC of the patients at the time of the IFD diagnosis
- To assess the adjunctive diagnostic methods used in the unit
- To describe the antifungal treatment used in the unit
- To evaluate malnutrition in IFD in children with cancer, and to compare the outcomes of children with malnutrition to those in children with normal nutritional status
- To evaluate the role of HIV in IFD in children with cancer – whether HIV-infected children are more susceptible to IFD than those who are HIV-uninfected and their outcomes
- To assess the overall outcomes of Paediatric oncology patients with IFD

2. Methodology

2.1 Study Design

A ten-year, single-centre retrospective, descriptive record review of paediatric patients with proven IFD while receiving treatment for cancer at the Paediatric Oncology Unit at CHBAH from 01 January 2011 to 31 December 2020, will be undertaken.

2.2 Study population

Paediatric patients treated at the CHBAH Paediatric Oncology Unit for any cancer with confirmed IFD.

2.2.1 Inclusion criteria

- Paediatric and adolescent patients aged 0 to 19 years.
- Cultures positive for a fungus from any normally sterile site (proven IFD cases only).
- One episode of IFD would be considered in a patient with multiple positive fungal cultures of the same organism per septic episode.

2.2.2 Exclusion criteria

- Patients treated for a presumed or probable IFD based on non-culture tests only.
- Patients treated for haematological conditions (for example aplastic anaemia) without an oncological diagnosis will also be excluded.
- Patients with records that are significantly incomplete.

2.3 Data collection

This is a retrospective study and data will be collected, with permission, from patient records. Patients fulfilling the inclusion criteria will be identified using the unit's electronic database, and their files will be extracted to collect data once ethics approval has been granted and permission from the hospital and unit have been obtained.

The data will be recorded onto a data collection sheet (see Appendix) with a study number – eliminating the need for personal details to keep participants anonymous. Data collected will be

entered onto an Excel database on a password-protected computer where no one other than the researcher will have access.

Data collected will include:

- Patient demographics – age, sex, racial group, socioeconomic status;
- HIV status;
- Anthropometry at the time of cancer diagnosis, and presence of malnutrition at the time of proven IFD diagnosis (classified according to the World Health Organization (WHO) Growth Standards);
- Type of malignancy, stage of disease;
- Therapy: chemotherapy, surgery, radiation therapy;
- Use of high dose corticosteroids;
- Presence of indwelling catheters – chemotherapy ports or central venous catheters, urine catheters;
- Duration of hospital stay and use of antibiotics (including duration and empiric versus targeted prior antibiotic therapy);
- Full blood count with a white cell differential count including the absolute neutrophil and lymphocyte counts at the time of proven IFD diagnosis, categorising the ANC into profound, severe and moderate (see definitions below), as well as the duration of the neutropenia prior to the diagnosis of IFD;
- Use of TPN prior to the IFD diagnosis;
- Presence or absence of abdominal surgery at the time of IFD diagnosis;
- Fungal infection:
    - Patient's presentation – pneumonia, fever, urinary tract infection, meningism, other;
    - Organism;
    - Susceptibility of the organism;
    - Site cultured;

- Markers of infection – BDG, GM, C-reactive protein (CRP) or procalcitonin (PCT) at the time of IFD diagnosis;
- Antifungal therapy used

- Patient outcomes.

2.4 Data analysis

Categorical or binary data will be presented as proportions (i.e. percentages), and analysed using the Chi-square test, or Fisher's exact test, as appropriate. For continuous variables, the difference in means between groups with normally distributed data will be analysed with the Student t- test. Non-parametric tests will be used to compare the medians of non-normally distributed continuous data.

Logistic regression will be used to compare the characteristics of IFD and outcomes of children with 1) malnutrition and those without malnutrition; 2) HIV infection and those without HIV infection; 3) TB (either confirmed or suspected) and those without TB. Logistic regression will be conducted using univariate analysis, and variables associated with P-values of <0.20 in groupwise comparisons will be included in multivariate logistic regression models to evaluate risk factors for IFD in each of these stratified analyses.

P-values of < 0.05 will be considered statistically significant in all tests.

2.5. Confidentiality and ethical considerations

Patient confidentiality will be maintained throughout the study. Patient data will be anonymised by the assignment of unique study numbers. A linking log of patient names and numbers will only be available to the study principal investigator and supervisors. Information will be kept on a secure computer that is password-protected. As this is a retrospective data analysis study, we will request a waiver of informed consent through the Human Research Ethics Committee (Medical) of the University of the Witwatersrand.

Application for the study will also be made to the University of the Witwatersrand Faculty Graduate Studies Committee, the CHBAH Department of Paediatrics and Child Health, the CHBAH Medical Advisory Board, and the National Health Laboratory Service (NHLS) Ethics Committee.

2.6. Potential Limitations

Incomplete medical records may be a limiting factor in collecting all required data. Fungal infections can be present without a positive culture, which would increase the number of false negative invasive fungal disease therefore reducing the sample size of the study. The unit does not have routine access to broncho-alveolar lavage, one of the tests often used to diagnose fungal infections (specifically Aspergillosis), which would also contribute to a smaller sample size of positive cultures.

2.7. Projected Outline

2.7.1 Timeline

2020										2021		
Month	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Feb	Mar	Apr
Literature review	X	X										
Protocol preparation			X	X	X							
Protocol assessment and revision						X	X	X	X	X	X	X
Ethics application												X

	2021								2022
	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan
Ethics application	X	X	X						
Data Collection				X	X	X	X		
Data Analysis								X	X
Write Up for Submission									X

2.8. Costs and funding

Costs will include printing of data collection sheets, the study protocol and final manuscript.

At roughly R1.00 per page, printing costs may exceed R2500.00 depending on the number of patients enrolled in the study and the length of the dissertation.

No other costs should be incurred. The Faculty of Health Sciences will be approached to assist with funding of publication costs (estimated at R 16,000.00).

2.9. Definitions

2.9.1 Anthropometry

Severe acute malnutrition (SAM)

- Weight-for-height less than -3 standard deviations below the mean according to the WHO Growth Standards;
- Mid-upper arm circumference (MUAC) less than 115 mm in children older than 6 months of age;
- Nutritional oedema.

Moderate acute malnutrition (MAM)

- Weight-for-height between -3 and -2 standard deviations below the mean according to the WHO Growth Standards;
- MUAC 115-125 mm in children older than 6 months of age.

2.9.2 Fever

A temperature of 38.3°C or above as a single reading, or more than 38°C sustained for over one hour.

2.9.3 Neutropenia

- Profound: ANC of ≤100 cells/mm^3.
- Severe: ANC of 101 to ≤500 cells/mm^3.
- Moderate: ANC of 501 to ≤1000 cells/mm^3.
- Prolonged: ANC ≤1000 cells/mm^3 for seven days or more.

2.9.4 Sterile site

Blood, cerebrospinal fluid, urine, joint fluid, pericardial fluid, pleural or ascitic fluid as well as tissue obtained on biopsy collected under sterile conditions are considered sterile.

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4. Appendix

Data collection sheet

Variable	Variable Code	Result	
Study No	patid		
Name	name		
Surname	Surname		
Hospital no	hosp_no		
Racial group	race		
Socioeconomic status	ses		
Sex	sex		M=male; F=female
Date of birth	dob		dd/mm/yyyy
Date of admission	doa		dd/mm/yyyy
Date of cancer diagnosis	ddx		dd/mm/yyyy
Date of IFD diagnosis	d_ifd		dd/mm/yyyy
HIV status	hiv		
If HIV positive:			
CD4 count	cd4		
CD4 Percent	cd4_per		
Date of CD4 collection	cd4_date		dd/mm/yyyy
HIV VL	hivvl		dd/mm/yyyy
Date of HIVVL collection	hivvl_date		dd/mm/yyyy
Date of ART start	d_art		dd/mm/yyyy
ART regimen	art_reg		Abbreviations: ABC = abacavir; 3TC = lamivudine; EFV = efavirenz; D4T = stauvdine; LPV/r = lopinavir/ritonavir
Anthropometry			
Weight	wt		kg
Wt/age (Z score)	wfa		
Height	ht		cm
Ht/age (Z score)	hfa		
Wt/Ht (Z score)	wfh		
MUAC	muac		cm
Malignancy			
Type	malig_type		
Stage	malig_stage		
Therapy			
Chemotherapy	chemo		y=yes; n= no
Surgery	surg		y=yes; n= no
Radiation	radx		y=yes; n= no
High dose corticosteroids	hdcort		y=yes; n= no
Indwelling catheter	ind_cath		y=yes; n= no
Chemotherapy port	chemo_port		y=yes; n= no
Central venous catheter	cvc		y=yes; n= no
Urine catheter	urc		y=yes; n= no
TPN use	tpn		y=yes; n= no
Antibiotic	abic		y=yes; n= no
Duration of antibiotic	abic_durn		days
Abdominal surgery	surg_abd		y=yes; n= no
Tuberculosis	tb		y=yes; n= no
If TB, date of TB Rx start	d_tb_start		dd/mm/yyyy
Certainty of TB Dx	tb_class		C = confirmed; P = probable
If TB, date of TB Rx completion	d_tb_end		dd/mm/yyyy
FBC			
WCC	wcc		x 109/L
Hb	hb		g/dl
MCV	mcv		fl
Plt	plt		x 109/L
Lymphocyte count	lymph		cells/mm3
ANC	anc		cells/mm3
Date of FBC	d_fbc		dd/mm/yyyy
Degree of ANC suppression	anc_cat		P = profound; S = severe; M = moderate
Duration of ANC suppression	anc_durn		days

Variable	Code	Result	
Fungal infection			
Patient presentation			
Fever	fever		y=yes; n= no
Pneumonia	pneum		y=yes; n= no
UTI	uti		y=yes; n= no
Meningitis	menin		y=yes; n= no
Abdominal symptoms	abd_sx		y=yes; n= no
Skin lesions	skin		y=yes; n= no
Mucositis	muco		y=yes; n= no
Other	other		
If other, please explain			
Fungal organism	fung_id		
Site cultured	fung_site		
Date of fungal culture	d_fung_cul		dd/mm/yyyy
Date of culture positivity	d_fung_pos		dd/mm/yyyy
Time to positivity	time_pos		hours
Susceptibility pattern			
Fluconazole	fluc		S = susceptible; I = intermediate; R = resistant
Itraconazole	itra		S = susceptible; I = intermediate; R = resistant
Amphotericin B	amph		S = susceptible; I = intermediate; R = resistant
Voriconazole	vori		S = susceptible; I = intermediate; R = resistant
Micafungin	mica		S = susceptible; I = intermediate; R = resistant
Other	rx_other		S = susceptible; I = intermediate; R = resistant
If other, please explain			
Histology	hist		y=yes; n= no
Date of histology	d_hist		dd/mm/yyyy
Yeasts seen	yeast_obs		y=yes; n= no
Hyphae seen	hyph_obs		y=yes; n= no
Tissue invasion seen	invas_obs		y=yes; n= no
Granulomata seen	gran_obs		y=yes; n= no
Other findings on histo	histo_oth		y=yes; n= no
If other, explain			
Markers of infection			
BDG	bdg		pg/ml
Date BDG	d_bgd		dd/mm/yyyy
GM	gm		odi
Date GM	d_gm		dd/mm/yyyy
CRP	crp		mg/L
Date CRP	d_crp		dd/mm/yyyy
PCT	pct		ug/L
Date PCT	d_pct		dd/mm/yyyy
Antifungal therapy	fung_rx		y=yes; n= no
Antifungal 1	af_1		
Duration Antifungal 1	af_1_durn		days
Antifungal 2	af_2		
Duration Antifungal 2	af_2_durn		days
Antifungal 3	af_3		
Duration Antifungal 3	af_3_durn		days
Patient outcome	outcome		
Outcome date	dod		dd/mm/yyyy