FUNGAEMIA IN THE NEONATAL UNIT AT CHRIS HANI BARAGWANATH HOSPITAL: RISK FACTORS, AETIOLOGY, SUSCEPTIBILITY TO ANTIFUNGALS AND OUTCOME.

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of

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DECLARATION

I, Firdose Lambey Nakwa declare that the research report is my own work. It is being submitted for the degree of Master of Medicine in the branch of Paediatrics in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

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___________day of ______________, 2011.

I, certify that the study contained in this thesis has the approval of the Committee for Resarch in Human Subjects of the University of the Witwatersrand, Johannesburg. The ethics clearance number is M110848 (which incorporates the original clearance number M03-10-38)

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___________day of ______________, 2011.

DEDICATION

I thank my parents Mohamed Rashid Lambey Nakwa and Zulagha Nakwa, my siblings Mohamed Faried Lambey Nakwa, Zainab Lambey Nakwa, Ismail Lambey Nakwa and Mahmood Lambey Nakwa; and my delightful nephew Rayhaan Lambey Nakwa for their understanding and endless support in my quest to complete this research report.

PRESENTATIONS

Nakwa FL, Velaphi SC, Wadula J, Khoosal MM. Fungal sepsis in a neonatal unit. Conference Proceedings of the Priorities in Perinatal Care; 2006 Mar; Drakensberg, Kwazulu Natal.

ABSTRACT:

TITLE: FUNGAEMIA IN THE NEONATAL UNIT AT CHRIS HANI BARAGWANATH HOSPITAL: RISK FACTORS, AETIOLOGY, SUSCEPTIBILITY TO ANTIFUNGALS AND OUTCOME.

Aim

The aim was to determine the epidemiology of invasive fungal infections at Chris Hani Baragwanath Hospital. The specific objectives were to determine the 1) risk factors, 2) clinical presentation, 3) laboratory abnormalities, 4) organisms and their susceptibilities and 6) outcome in neonates with positive blood or CSF fungal cultures at Chris Hani Baragwanath Hospital.

Methods

This was a retrospective record review of patients who had positive blood or CSF cultures. Patients were identified by a computerized microbiological surveillance database. The data was collected over a three-year period from January 2002 to December 2004. Patient hospital files were reviewed for clinical signs, full blood count (FBC), C-reactive protein (CRP) and outcomes. Fungal culture results were reviewed for susceptibilities. To identify risk factors a convenient cohort was compared to the patients with fungal sepsis. The data was analysed using a Statistica software package.

Results

There were 150 patients with fungal sepsis among admissions over this 3 year-period giving an incidence of 1.3 per 100 admissions. Thirty-nine records were not found thus 111 patient records were reviewed. The median birthweight was 1280g and the gestational age 30 weeks. The median age of onset was 16 days and 6.3% had early onset fungal sepsis. There were 61 males. Twenty-eight percent of patients were born to HIV positive mothers. *Candida parapsilosis* was the commonest (56%) organism isolated followed by *C. albicans* (43%). All the *C. albicans* isolates and 93% of the *C. parapsilosis* isolates were susceptible to amphotericin B. Fluconazole susceptibilities were reported as, 96% for *C. albicans,* and 60% of the *C. parapsilosis* as being susceptible. Central venous catheters (CVCs) (p=<0.001), the use of TPN (p=<0.001) and third generation cephalosporins were identified as risk factors associated with fungal sepsis. The all-cause mortality and *Candida*–related mortality were 30% and 23% respectively. The non-survivors had lower platelet counts (p=0.007) than the survivors. Patients with Gram-negative sepsis had lower platelet counts than the fungal group (p=<0.001) on the repeat laboratory parameters.

Conclusion

The incidence is 1.3 per 100 admissions. Risk factors associated with fungal sepsis are very low birthweight and gestational age, the use of TPN, CVCs and third generation cephalosporins. *Candida parapsilosis* is the common organism causing fungal sepsis in neonates. *Candida albicans* was associated with a higher mortality. Thrombocytopenia is not organism specific to fungal sepsis.

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LIST OF ABBREVIATIONS

ABCD	Amphotericin B Colloid Dispersion
ABLC	Amphotericin B Lipid Complex
AIDS	Acquired Immunodeficiency Syndrome
CRP	C-reactive protein
CSF	Cerebrospinal Fluid
CVC	Central Venous Catheter
ELBW	Extremely Low Birthweight
EOS	Early - onset Sepsis
ETT	Endotracheal Tube
FBC	Full Blood Count
GIT	Gastrointestinal tract
GN	Gram-negative
HCW	Health Care Workers
HIV	Human Immunodeficiency Virus
IDSA	Infectious Diseases Society of America
IgG	Immunoglobulin G
IL-6	Interleukin 6
IL-8	Interleukin 8
L-AmB	Liposomal Amphotercin B
LOS	Late – onset Sepsis
MDG	Millineum Development Goals
MIC	Minimum Inhibitory Concentration
NAC	Non-*albicans Candida*
NEC	Necrotising Enterocolitis

NICHD	National Institute of Child and Human Development
NICU	Neonatal Intensive Care Unit
R	Resistant
S	Susceptible
S-DD	Susceptible Dose Dependent
TPN	Total Parenteral Nutrition
USA	United States of America
UTI	Urinary Tract Infection
VLBW	Very Low Birthweight
WHO	World Health Organization

PREFACE

Advances in neonatal care have escalated dramatically. With the VLBW infant surviving longer and having prolonged hospital admissions, *Candida* is emerging as an important pathogen. *Candida* is associated with a significant morbidity and mortality. This study was undertaken as I had noticed a trend towards an increase in the number of yeasts being isolated in the unit. A study to look at the epidemiology of *Candida* had not been undertaken in the neonatal unit in the Chris Hani Baragwanath Hospital. Hence, it was imperative to outline the patient characteristics, risk factors, susceptibilities and outcome of the patients with *Candida* sepsis. The information attained would assist us as clinicians to improve the quality of care and the current management of patients with *Candida* sepsis

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CHAPTER 1

LITERATURE REVIEW

1.1 Background

The World Health Organization (WHO) reports that 10 million children under the age of 5 years die each year.[1] The mortality rate of children under the age of 5 years in Sub-Saharan Africa is 164 per 1000 and in South Africa is 67 per 1000 live births.[2] Neonatal deaths accounts for 30-40% of these under 5 deaths.[1] In Sub-Saharan Africa the neonatal mortality rate was reported to be 42-49 per 1000 live births in the year 2000. In the developing world 1.6 million of neonatal deaths are due to neonatal infections.[1] One of the Million Development Goals (MDG) is to reduce under-5 mortality rate by 66% by the year 2015.[3] Therefore, as part of the process of achieving this goal of reducing neonatal deaths especially those due to neonatal infections; it is important to understand the epidemiology of neonatal infections. This would include identifying the risk factors and causes of these infections.

Neonatal infection can be classified as congenital or acquired. In the acquired group it can be sub-divided into early-onset sepsis (EOS) (infections occurring within the first 72 hrs of life) and late-onset sepsis (LOS) (infection occurring after 72 hrs in hospital.[4,5] Sepsis is defined as the isolation of an organism from a blood culture taken from a neonate with signs and symptoms of an infection.[5,6] The signs of infection are often non-specific during the neonatal period. The diagnosis of sepsis is often made on clinical suspicion. The National Institute of Child and Human Development (NICHD) Neonatal Network and other studies have reported that the most common signs of sepsis are apnoea (55%), gastro-intestinal tract (GIT) symptoms (46%), increased need for oxygen and ventilatory requirements (36%), lethargy and hypotension (25%).[5,7-9] The blood culture is the gold standard for the diagnosis of sepsis.[4] The blood culture is not as sensitive for fungal elements as it is for bacterial pathogens therefore; fungal elements take longer to culture[10]. The full blood count (FBC), C-reactive protein (CRP), Interleukin 6 (IL-6), Interleukin 8 (IL-8) and procalcitonin are other useful laboratory tests that are used to assist in diagnosing infection.[4,11]

Infections due to invasive fungal infections account for 15% of all blood stream infections (BSI) in the neonatal intensive care unit (ICU).[12] *Candida albicans* is the most frequently recovered fungus, but in the last two decades the trend has changed with *C. parapsilosis* as the more prevalent organism. As invasive candidemia is associated with an attributable mortality of 38% and a crude mortality of 30%-75%; emphasis has been placed on identifying risk factors and empirically treating patients whilst awaiting results.[10] In this review and report I am focusing mainly on *Candida* infections, looking at the pathogens, presenting signs and symptoms, risk factors, treatment and mortality associated with *Candida* infections.

1.2 *Candida* species causing infections in neonates

Candida is the genus commonly isolated in neonates. *Candida* organisms are yeasts and exist in a unicellular form. They are ovoid and thin-walled and reproduce by budding.[13,14] *Candida* organisms grow well in blood culture bottles and agar and do not require special fungal media. They form smooth, creamy white glistening colonies and resemble staphylococcal colonies.[15] To further identify the organism as a yeast, one can facilitate germ tube formation by placing the organism in serum and within 90 minutes germ tubes are formed. Metabolic tests can be utilized to speciate the organism.[15,16] This is based on physiologic parameters rather than morphologic characteristics. They stain as gram positive on haematoxylin and eosin stains. Potassium hydroxide (10%) solution can be used to facilitate hyphae and pseudohyphae formation.[15,16,17]

Candida species is a normal commensal in humans. It is commonly found on skin, throughout the gastrointestinal tract (GIT), in sputum and the female genital tract.[16,17] *Candida albicans* is found in soil, in animals, in hospital environments, inanimate objects, and food. The non-*albicans Candida* species live in animal and non-animal environments.[18] *Candida* species isolated from blood or sterile fluid (cerebrospinal fluid, pleural and peritoneal fluid, and urine aspirates) should be considered a pathogen regardless of whether the host is immunocompetent or immunodeficient. Rarely is *Candida* species a laboratory contaminant.

There are more than 150 *Candida* species but only 15 cause disease in humans.[14,19] These include *C. albicans*, *C. parapsilosis, C. glabrata, C. tropicalis, and C. krusei, C. guilliermondii, C. dubliensis, C. rugosa and C. lusitaniae. Candida albicans* has been reported in many studies as the commonest Candida species. *Candida parapsilosis, C. glabrata, C. tropicalis, and C. krusei* are among the common non-*albicans Candida* (NAC) species. *Candida guilliermondii, C. dubliensis, C. rugosa and C. lusitaniae* are the less common NAC species.[20] Ninety-seven percent (97%) of candidemia is caused by *C. albicans, C. parapsilosis, C. tropicalis, C. glabrata, and C. krusei*.[15,16,18]

In neonates the two commonest causes of candidaemia are *C. albicans and C. parapsilosis.* In the last two decades there has been a decrease in *C. albicans* and a proportional increase in the non-*albicans Candida* (NAC) species causing invasive disease.[20-22] Kosshoff et al reported a 60% increase in the prevalence of *C. parapsilosis* over a 5-year period 1991-1995.[23] The reasons for the shift in an increase in NAC include the use of prophylactic azoles, the HIV epidemic and the use of fluconazole for oropharyngeal and oesophageal candidiasis.[22,24] In Pfaller's study a geographical as well as an interhospital variation with respect to species isolation and a susceptibility pattern was found.[25-27] In the USA and South America, Pfaller reported that *C. albicans* isolates (53.3%) were more prevalent than NAC (43.8%) differing from Canada where 50% of all the isolates were due to NAC.[25] Of the NAC isolates in Canada, *C. parapsilosis* was the commonest *Candida* species isolated. There was also a difference in species isolated from different groups of hospitalized patients. In neonates the commonly isolated fungi are *C. albicans* and *C. parapsilosis*, whereas patients with haematological malignancies and those with solid tumors are often infected by *C. krusei*, and *C. tropicalis* and *C. glabrata* respectively.[14,22,26-28]

1.2.1 *Candida albicans*

Candida albicans is the most common species (50-70%) isolated in neonates; followed by *C. tropicalis, C. parapsilosis and C. glabrata*.[17,29] It is more virulent than *C. parapsilosis*. It is a normal commensal of the female genital tract and accounts for most of the early–onset fungaemia in the neonate. It is associated with a higher mortality (44.8%) compared to *C. parapsilosis* which has been reported to have a mortality rate of 28.5%.[30] This is accounted for by its virulence factors. Being a normal commensal of skin it stands to reason that any breach in the skin barrier leads to seeding and the development of invasive disease. It has a cell envelope that has molecular adhesions that bind it to host epithelial surfaces.[31] It has a rapid germination upon seeding into the bloodstream, secretes proteinase, form true hyphae, resists phagocytosis and adheres to host surfaces.[29]

It is an aerobic yeast that can exist anaerobically. It grows into two main forms budding yeast or as continuously extending hyphae with pseudohyphae as an intermediate form. It is naturally diploid and has no known sexual cycle. *Candida albicans* has been regarded as the dominant *Candida* species but in the last two decades is being replaced by *C. parapsilosis* as the common species in the neonatal population.

1.2.2 *Candida parapsilosis*

It is the second most common cause of fungal sepsis in neonates and infants.[32,33] The cells are oval, round or cylindrical in shape on Sabouraud's agar. The colonies are creamy, white, shiny, smooth or wrinkled.[34] *Candida parapsilosis* do not have true hyphae but pseudohyphae and can exist in the yeast form as well. It is a normal commensal of skin and its pathogenicity is limited by an intact skin barrier. It can spread nosocomially by hand carriage of health care workers.[35]

Its incidence varies with geographical region.[27] In the United Kingdom *C. parapsilosis* accounts for one quarter[36] of invasive fungal infections in ill neonates whereas it accounts for one third of bloodstream infections in the United States.[37] It has emerged as one of the predominant fungal pathogens in the NICU and is associated with an increased mortality. It can occur without prior colonization; it grows easily in total parenteral nutrition (TPN) and forms biofilms on catheters.[34,38,39]

Unlike *C. albicans, C. parapsilosis* organisms are not commonly found in the vaginal tract. The hands of health care workers (HCW) are a major vector coupled with poor hand hygiene and hand washing techniques.[27,32,35] The virulence factors include slime production. This causes cell surface hydrophobicity and co-adherence among yeast cells. This leads to aggregation on epithelial cells. They form biofilms on devices and this renders them impermeable to antifungals.[27,40] They secrete enzymes e.g. aspartic proteinases that facilitate invasion and colonization of host tissue; phospholipases that disrupts the host membrane, lipases that digest lipids for nutrition and adheres to host cells and tissues.[34]

1.2.3 *Candida krusei*

Candida krusei is one of the NAC that is increasing as a human pathogen. It is isolated from natural habitats and is a transient commensal in man. It has been isolated from mucosal surfaces (oral, GIT, vaginal and anorectal) in healthy individuals. It is commonly isolated from patients who are severely immunocompromised and who have haematological malignancies.[41,42] It is one of the three most common yeast combinations isolated with *C. albicans* in dual *Candida* infections. The other two being *C. glabrata* and *C. tropicalis*. It has the appearance of long grain rice. It grows on Sabouraud's agar as spreading colonies with a rough, whitish yellow surface. Its cell wall has low reactivity with antisera against other *Candida* species. It is less virulent than *C. albicans* and is weakly immunogenic. It is found in two basic forms, yeasts and pseudohyphae and they exist together in growing cultures. *C. krusei* can grow in vitamin-free media at a temperature of 43-45 degrees Celsius.

It is less invasive than *C. albicans. Candida krusei* do not adhere to and penetrate epithelial cell surfaces as well as *C. albicans*. It adheres more to inert surfaces than buccal mucosa and therefore has a predilection for implants and catheters. Its biofilm formation is more extensive than any other species because it has increased cell surface hydrophobicity and increased adherence to inert plastic. It does not produce hydrolytic enzymes. *Candida krusei* is more susceptible to killing by polymorphonuclear cells, macrophages and other immunoeffector cells than *C. albicans.*

1.2.4 *Candida glabrata*

The high prevalence of HIV and AIDS resulting in the long term use of fluconazole has lead to the selection of *C. glabrata* infection.[24,38,40,43,44] *Candida glabrata* like *C. krusei* is low in virulence. The lack of hyphae is a factor in this regard. It produces proteinase enzymes and has cell surface hydrophobicity that is similar to *C. albicans*. It does not possess the B-integrin binding receptor therefore; it is less adherent to epithelial cell surfaces than *C. albicans*. It does not exhibit phenotypic switching like *C. albicans*. Host defences do not have to be that stringent in combating *C. glabrata* because it is less virulent. Risk factors that predispose to *C. glabrata* infections include prolonged hospitalization and prior antimicrobial use and increased colonization in immunocompromised patients.[21] In addition to the other risk factors that predispose to *Candida* infections, there is an increase in incidence because of prior azole exposure.[22,38] It has become an important pathogen because it has decreased susceptibility to antifungal agents.[20] It causes infection in any organ and therefore has a diverse clinical picture. *C. glabrata* candidemia can present with a low grade fever to fulminant septic shock. It has a higher mortality than *C. albicans* but this is attributed more to host factors than virulence of the organism.[44]

1.3 Incidence

Fungal sepsis is becoming an increasingly important pathogen in the neonatal intensive care units.[45] It is the third most common cause of nosocomial bloodstream infection in the United States.[14] It affects up to 3000 infants each year in the United States.[19,46]

The frequency has increased by eleven fold in some institutions. In a study by Kossoff et al over a fifteen year period; from 1981 to 1995, the incidence had increased from 2.8% to 28%.[23] The NICHD Neonatal Network had also reported that the incidence of all late onset sepsis due to *Candida* had increased from 9% to 12%.[47,48] Chapman has observed the incidence to range from 2.2% to 12.9% in the very low birth weight (VLBW) infant and from 5.5% to 16.5% among the extremely low birth weight infant (ELBW).[49,49,50] An overall incidence of 12.3 per 1000 admissions has been reported.[7] The overall incidence varies from institution to institution.

1.4 Risk Factors

1.4.1 Intrinsic Factors

It has been well documented that very low birth weight infants are at increased risk of developing candidiasis due to the increase in the fungal burden or colonization in this population.[4,35,51] The incidence has been reported as 4-15% in the ELBW infant.[10,45,51,52] Gestational age has been reported to be inversely associated with *Candida* sepsis.[53]

As early as 1986 Bayley et al found that 7.7% of colonized infants developed systemic fungal disease.[54] A Taiwanese group documented a 22% fungal colonization rate within two weeks after birth and reported that one fifth of colonized infants will develop invasive disease. This is another risk factor for fungal sepsis.[7,55] Host factors such as immaturity of the immune system and the infant's fragile skin and mucous membranes play a role in the development of fungal sepsis.[35] The mucous membrane defenses are decreased in the VLBW infant. The breakdown of skin through the insertion of the central venous catheter per se is an independent risk factor.[14] Firstly there is a breach of the skin. Secondly, the organism forms a biofilm which surrounds the catheter. The *Candida* organism harbors itself within the biofilm thus protecting itself from the antifungals.

The neonate has an altered innate or adaptive immunity.[14] Serum complement levels, fibronectin, and defenses and cytokine production are decreased in all newborns; in premature babies to a greater degree than the term infants.[18] There is a limited production of antibodies in response to the invading pathogen. Cellular defenses such as chemotaxis, phagocytosis and microbiological killing are relatively impaired.[11] The use of intralipid as a feeding modality further decreases the chemotactic ability of the neonatal leukocytes. This renders the premature infant more susceptible to infections and leads to, dissemination and deep-seated tissue infections.[7] Maternal IgG transfer usually occurs in the third trimester and confers some immunity on the neonate.[11,56] Thus, in a premature baby there is a limitation on passively acquired immunity.[37]

1.4.2 Extrinsic Factors

The other risk factors include the use of H2 blockers, indomethacin, steroids and antibiotics especially carbepenems and third generation cephalosporins.[4,57,58] The introduction of foreign bodies like the use of instrumentation such as endotracheal intubation (ETT), central venous catheters (CVCs), umbilical venous or arterial catheters and nasogastric tubes in sick neonates are other risk factors.[17,35,52,53,59] The length of stay in the neonatal unit exposes these infants to nosocomial bacterial infections therefore increasing the need for venous access to administer antibiotics especially third generation cephalosporins.[31,60] Feeding intolerance and the use of intralipid solutions make these infants more susceptible to candidemia.[40,61] Total parenteral nutrition (TPN) mainly intralipid acts a good medium for fungal elements to grow. Abdominal surgery has also been identified as a risk factor.[32,51,52]

1.5 Clinical Presentation and Laboratory Findings

Patients with invasive *Candida* infections can present with non-specific signs of infection. The clinical manifestations range from temperature and glucose instability, respiratory distress, increase in oxygen requirements, features of necrotizing enterocolitis (NEC), feeding difficulties, lethargy, abdominal distension and vomiting.[17] This makes it difficult to identify whether the offending pathogen is a fungal organism or a bacterial organism. This often results in delays in starting the appropriate treatment.[57]

Among the laboratory parameters a high C-reactive protein and thrombocytopenia[62] are features that have been identified to be associated with *Candida* infections.[17,57] Positive blood cultures and cultures from other sterile sites are a gold standard to diagnose invasive *Candida* infections.[17]. However, blood culture has a low yield; between 50-80% for a positive fungal result.[14,19,57] The yield on the blood culture also takes a long time, as the organism is slow growing and may take a while before it becomes positive.[37] At least 30 % of neonates are diagnosed with invasive candidiasis at post mortem. Newer methods to diagnose fungal infections have been formulated and are currently being tested. This includes B-D glucan test, breath test, and growth on a special broth.

Because of the difficulties in making a diagnosis of fungal sepsis, Benjamin et al has developed a scoring system looking at the gestational age and other risk factors. The clinical predictive score is based on the probability of developing fungal infection when a patient has these risk factors.[10] A score of 0, 1 or 2 is assigned based on risk factors such as decreased gestational age, thrombocytopenia and the use of third generation cephalosporins. If the neonates were thrombocytopenic a score of 2 was assigned (otherwise 0), if they received a 3rd generation cephalosporins or a carbepenem a score of 1 was assigned (otherwise 0), if the gestational age was 25-27 weeks a score of 1 was assigned (otherwise 0), if the gestational age was <25 weeks a score of 2 was assigned. If the combined score was 2 or more it was regarded as a positive candidemia score. A score >2 has a sensitivity of 85% and a specificity of 47%. Once a patient meets these criteria antifungal treatment would be started empirically.

1.6 Treatment

The treatment modalities are limited but newer drugs are being formulated and there are some trials that are being conducted. The treatment is limited and is fraught with its own complications depending on the organism's sensitivity patterns, the patient's medical condition and the epidemiology of the strains in the NICU. Polyenes (Amphotericin B), azoles (fluconazole, voriconazole, itraconazole, miconazole) and fluorinated pyrimidines (flucytosine), echinocandins (caspofungin, micafungin) are among the armamentarium used to treat candidemia.[17,50,63] The antifungal agents most commonly used are amphotericin B and fluconazole, therefore the next two sections will review these two drugs.

1.6.1 Amphotericin B

The gold standard of therapy for candidiasis is amphotericin B.[17] It was first formulated in the 1950s. Amphotericin B is fungicidal. It binds to the ergosterol component of the fungal cell wall; this then leads to membrane leakage and eventually cell death.[50,64] Its use is limited by its safety profile. It causes nephrotoxicity and hepatotoxicity. It is often used empirically for suspected systemic candidiasis.[63]

It is sometimes used in combination with fluconazole for treating refractory candidiasis.[65] Some studies show antagonism between the two drugs but clinically these drugs are synergistic with a positive reponse.[63,66,67] It is also used in combination with flucytosine to treat meningitis due to candidiasis.[65]

Amphotericin B comes in different forms namely Amphotericin B lipid complex (ABLC), Amphotericin B colloid dispension (ABCD), liposomal Amphotericin B (L-AmB). These lipid formulations have similar efficacy and a better side-effect profile than the standard amphotericin B.[30,68-70] The other advantage is that higher doses can be used.[71,72] Their use has been advocated in renal failure and when there is failure of standard amphotericin B treatment.[30,50,73,74] Lipid formulations are better if given as first line antifungal treatment and at high doses.[17] Study by Linder has demonstrated a shorter duration of antifungal therapy with lipid formulations and that there is a better eradication of the organism.[60] Liposomal AmB has the greatest renal protection of the three lipid formulations.[63,70] Despite its high side-effect profile, amphotericin B is the gold standard of treatment for invasive candidiasis.[63,75]

1.6.2 Fluconazole

Fluconazole is an azole that was developed in the 1970s. It acts by inhibiting CYP-dependent 14-α-demethylase.[50] This inhibition interrupts the conversion of lanosterol to ergosterol. Ergosterol is a major component of the fungal cell wall.[64] This then increases membrane permeability and leads to cell leakage and eventually cell death. It also leads to cell growth inhibition, morphologic changes, cessation of sterol synthesis, and reduction in adhesion to epithelial cells. Fluconazole is fungistatic and not fungicidal. It has good penetration into cerebrospinal fluid (CSF), liver, kidney and spleen. It is excreted unchanged in the urine therefore it is a good agent for treating UTI (urinary tract infections). Fluconazole is as effective as amphotericin B and it has less toxicity therefore is a safer alternative to amphotericin B in treating candidiasis.[76] Driessen et al has reported that fluconazole has the same efficacy in clearing the infection as amphotericin B.[77] Some centers use fluconazole as a first line agent as empiric therapy because of its low side effect profile and excellent oral bioavailability.[78]

Most *Candida* species are susceptible to fluconazole, but *C. glabrata* is dose-dependent and *C. krusei* is resistant.[78-80] However, resistance to fluconazole has been reported. This is due to the HIV epidemic which has resulted in the widespread use of fluconazole to prevent and treat candidiasis. This has lead to more non-*albicans Candida* (NAC) species such as *C. glabrata* and *C. guilliermondii* becoming resistant to fluconazole.[21,40,43] Fluconazole has been shown to reduce the incidence of colonization and invasive candidemia when used as a prophylactic agent.[81-84]

There has been a concern in the emergence of *Candida* strains (*C. krusei* and *C. glabrata*) that exhibit resistance to fluconazole.[85] This has been thought to be due to the use of fluconazole for prophylaxis in neonates at risk of invasive fungal sepsis. Many studies have documented no resistance to fluconazole in patients that have received prophylaxis.[81-83] Kauffmann et al has speculated that with the lower dose and less frequent dosing schedule resistance may be prevented.[83] However, resistance can develop over time in a population that has received fluconazole.[60,86]

A recent Cochrane meta-analysis has shown a decrease in fungal infections with fluconazole prophylaxis in VLBW infants.[87,88] Recommendations are that high risk populations should receive prophylaxis. The low dose and shorter dosing schedule should preclude from the development of resistant strains. Caution should still be exercised as the threat of resistance is ever looming.

Antifungal prophylaxis decreases the fungal burden and thereby decreases the incidence of colonization and invasive candidiasis.[89] Manzoni and others had shown that this strategy is effective in decreasing invasive *Candida* infections in the neonatal population.[84] However, the development of NAC strains and resistant *Candida* strains is still an ever looming problem.[21]

1.6.3 Combination therapy

Amphotericin B and flucytosine has been the first combination used for meningitis, endocarditis, and peritonitis caused by *Candida*.[90] The use of fluconazole and amphotericin B has shown antagonism in vitro but clinically these drugs are synergistic with a positive response.[63,65] A study by Rex et al has shown favourable outcomes.[91] There was a success rate of 68% vs 56% when amphotericin B and fluconazole were used in combination than when fluconazole used alone. The mortality was the same in both groups. Thus, amphotericin B and fluconazole are used in combination to treat refractory candidiasis. There is very limited data on the use of echinocandins in combination with amphotericin B.[65] It should have an additive effect and synergistic effect as caspofungin inhibits the cell wall synthesis and enhances the access of amphotericin B to its target which is the cell membrane.[92]

1.6.4 Empiric therapy

Fungal sepsis is a leading cause of mortality and morbidity but this is organism specific. Empiric therapy has been shown to decrease the mortality and morbidity in at risk patients. Most of the studies have been in adult cancer patients with a febrile illness and neutropenia.[93] The choice of antifungals range from amphotericin B to voriconazole. Amphotericin B is the most efficacious empiric antifungal used but it is quite toxic.[94] Comparative studies have shown that the efficacy decreases with the other classes of antifungals but the side effect profile improves. Kaufman and Fairchild have identified high-risk ELBW infants with risk factors such as CVCs, ETT in-situ, platelet count <100 000/mm^3, broad spectrum antibiotics, exposure to carbepenems and cephalosporins and a gestational age less than 28 weeks as a subset of patients that would benefit from empiric therapy.[7,38,95,96] A *Candida* clinical predictive model has been devised to assist in commencing empiric therapy as fungal elements take a long time to grow.[10] The duration of empiric treatment would be for as long as the blood culture is formally interpreted as negative i.e. if a blood culture is regarded as negative after seven days then empiric therapy would be continued for seven days.[7] In some institutions, if a patient is not responding to antibacterial treatment, and has one or more of the risk factors and/or features suggestive of a fungal infection; antifungal therapy would be commenced empirically. The choice of drug would depend on the epidemiology of *Candida* isolated in the NICU, the patient population and the medical condition of the patient[10] and whether the patient received fluconazole prophylaxis or not. If fluconazole prophylaxis is practiced in the unit then an antifungal other than fluconazole should be commenced as empiric treatment. Cross resistance as well as co-resistance to azoles are confounding variables. Non-*albicans Candida* isolates are also emerging as important pathogens[21] thus, the choice an antifungal as empiric therapy may be difficult.

1.7 Susceptibility of *Candida* Species to Antifungals

1.7.1 Susceptibility to Amphotericin B

Susceptibilities are carried out by detecting the minimum inhibitory concentrations (MIC) of the particular organism to an antifungal.[97] The MIC is defined as the lowest concentration of amphotericin B and fluconazole to reduce the turbidity of cells to >95% and 50%. For fluconazole if the MIC < 8ug/l the organism is classified as susceptible(S); and if the MICs are > 64ug/l the organism is classified as resistant (R). If the organism has an MIC between 16 and 32 ug/l it is classified as susceptible dose-dependent (S-DD), which means that at a higher dose of the antifungal the organism would be rendered dead. In the case of amphotericin B an MIC of <1ug/l is susceptible (S) and resistant as an MIC >2 ug/l.[96,97,98]

Ninety-five percent (95%) of *C. albicans* isolates are susceptible to amphotericin B, whilst 97-98% of *C. parapsilosis* isolates are susceptible.[95,99] Clerihew has reported that all *C. parapsilosis* isolates in her study are sensitive to amphotericin B.[36] *Candida glabrata* and *C. krusei* isolates are relatively resistant to the amphotericin B.[25,95] These two organisms have higher MICs for amphotericin B[67] and thus, the IDSA guidelines recommends a higher dosage of amphotericin B when treating them.[63,66] Yang and Shoa in Taiwan and Almirante in Spain have reported similar findings.[79,100,101] It has also been reported that there is an emerging resistance of *C. tropicalis* and *C. guillermondii* to amphotericin B.[38,99,102], whilst *C. lusitaniae* isolates are resistant to amphotericin B.[78]

1.7.2 Susceptibility to Fluconazole

Most *C. albicans* isolates are sensitive to fluconazole[103,104] whereas the NAC tend to have higher resistance rates to fluconazole.[25,43,85,102,105] In China Ying Liang Yang et al found that all *C. parapsilosis* isolates were sensitive to fluconazole and the *C. krusei* isolates having had the highest resistance rate to fluconazole.[79,106] Nine percent (9%) of *C. glabrata* and 100% of *C. krusei* isolates have been reported to be resistant to fluconazole.[25,31,104] Newer triazoles have been developed but the *C. glabrata* isolates had shown cross resistance to the triazoles hence, amphotericin B was used as therapy for this isolate. With the increased use of amphotericin B the resistance rates of *C. glabrata* isolates were reported as high as 20-36% in North America. Co-resistance to amphotericin B and fluconazole by this isolate may be an impending problem.[79] Thus, it is wise to monitor surveillance patterns so that emerging co-resistance can be identified and an appropriate antifungal is chosen as empiric therapy.

Azole prophylaxis has been implicated as causing a shift towards an increase in *C. krusei* and *C. glabrata* infections.[21,100,103,107] These strains are known to be inherently resistant to fluconazole. Whilst the overall incidence of candidemia is decreasing as azole prophylaxis decreases the rate of *C. albicans* infections there is an increase in infections with NAC species.[40] There should be an awareness when using fluconazole as empiric therapy in centers where fluconazole is used as a prophylactic agent.[104]

Sarvikivi has shown the emergence of a resistant *C. parapsilosis* strain to fluconazole.[86] This had occurred in patients in Finland that received fluconazole prophylaxis at the time that the blood culture was taken. This is in contrast to other studies that have proven that *C. parapsilosis* remains sensitive to fluconazole despite the patients having received prophylaxis. Therefore one needs to bear in mind that resistance can develop over time.

1.8 Mortality

The mortality rate is 20 – 40% and it depends on the *Candida* species.[8,9,14,47,51,57,63] The highest mortality is reported as being due to *C. albicans* among those with fungal sepsis.[30,47,75] This was noted by Faix in a single centre study with *C. albicans* accounting for 24% of the deaths and while there were no deaths in those with *C. parapsilosis*.[108] The NICHD Neonatal Network had also reported a significantly higher mortality (44%) due to *C. albicans* compared to 16% of those with *C. parapsilosis*.[47] This is also attributed to the timing of the infection. *Candida albicans* is vertically transmitted causing sepsis early in the more compromised host and *C. parapsilosis* causing infection in the older more immune competent host.[7-9,109] The crude mortality of *C. glabrata* and *C. krusei* is reported as 50% and 100% respectively[110]. In a study by Pappas and Rex the *Candida*-specific mortality in patients <13years for *C. glabrata* was 13% for *Candida*-specific mortality and 0% for *C. krusei*. This study also found that the survival rates were not any worse for *C. glabrata* isolates as compared to the other *Candida* species. If the patients with *C. glabrata* isolates were not treated it was associated with a lower survival rate compared to those who received treatment.[111]

CHAPTER 2

2. OBJECTIVES AND METHODS

2.1 Objectives

The objective for this study was to determine the epidemiology of invasive culture proven fungal infections at Chris Hani Baragwanath Hospital.

The specific objectives were to determine

1. the risk factors associated with fungal sepsis
2. clinical presentation of neonates with fungal sepsis
3. laboratory abnormalities in infants with fungal sepsis
4. the *Candida* species causing infections in neonates,
5. Susceptibilities of *Candida* species to commonly used antifungal agents
6. the mortality rates among neonates infected with *Candida*

2.2 Methods

2.2.1 Study Design

This was a retrospective record review. Patients were identified by a computerized microbiological surveillance database. Once identified the patient records were retrieved and analyzed. The data was collected over a three year period from January 2002 to December 2004. To identify the risk factors a control group was a convenient sample which was from records of patients who had culture proven Gram negative (GN) sepsis collected over the same time period.

2.2.2 Study Population

Patients who had positive blood and /or CSF cultures were identified through looking at the laboratory database. Patient's hospital records were retrieved for data collection. The data was collected with the aid of a data collection sheet (Appendix A). This sheet included section 1 which concentrated on both infant and maternal demographic data; section 2 dealt with the infective demographics and section 3 was dedicated to the infants' presentation at the time of sepsis. This included details such as the laboratory parameters full blood count (FBC), C-reactive protein (CRP) and blood culture, and cerebrospinal fluid (CSF) results both at the time of presentation and repeat results. Repeat results were the very next laboratory parameters (FBC, CRP blood and CSF culture) sampled from the same patient around the time of infection. It also included details of antibiotic usage, the use of central lines, and total parenteral nutrition. The details of the organism and its susceptibilities were also collected. Data on the outcome that is whether the patient died secondary to fungal sepsis was collected. Death due to fungal sepsis was defined as a patient having demised within two weeks of the positive fungal culture. Antifungals that were used in the unit during this period were amphotericin B and fluconazole. Ethics approval was obtained from the University of the Witwatersrand Committee for Research of Human Subjects.

2.2.3 Determining Risk Factors

To determine the risk factors for the development of fungal sepsis, data from infants who were infected with Gram-negative organisms were used for comparison. The Gram-negative cohort was a convenient sample as data had already been collected for another study.

2.2.4 Statistical Analysis

The information was entered onto a computer database and analyzed using the Statistica statistical software package. Mean and standard deviations were used to describe data with normal distribution and medians and 25th and 75th percentiles were used to describe data without normal distribution. Comparison between categorical variables was performed using the Chi-squared or Fisher exact test. Comparison between continuous variables was performed using a Students' t-test when there was a normal distribution otherwise a Mann-Whitney U test was used. A significant difference was taken as a p-value of less than 0.05.

CHAPTER 3

3. RESULTS

3.1 Incidence

One hundred and fifty (150) patients were identified to be infected by *Candida* from January 2002 to December 2004. There were 11589 admissions to the neonatal unit over this three year period. This gave an incidence of 1.3 cases of *Candida* per 100 admissions. Patient hospital records of 39 patients were not found leaving 111 infants for analysis.

3.2 Characteristics

Characteristics of the 111 infants that had their hospital records reviewed are shown in Table 3.1. Ninety percent of the patients reviewed were low birthweight (LBW) and premature, with a median birthweight of 1280g and a gestational age of 30 weeks. Seventy-three percent (81/111) of the patients were VLBW (weighing<1500g). Fifty-nine percent (59%) of the patients were born vaginally and in 93 patients (84%) the maternal HIV status was known. Of those with known HIV status 28% were born to mothers who were HIV positive. The median age of onset of patients who had positive *Candida* cultures was 16 days, and 6.3% of patients were less than 3 days old.

Table 3.1 Characteristics of Infants with Fungal Sepsis

Birth Weight (g)*	1280 (660-3300)
Gestational Age (weeks)*	30 (23-40)
Mode of delivery	
Vaginal	66 (59%)
Abdominal	35 (32%)
Maternal HIV Status	
Positive	31 (28%)
Negative	62 (56%)
Unknown	18 (16%)
Apgar	
1 minute	7 (1-10)
5 minutes	8 (4-10)
Gender (n=111)	
Female	49 (44%)
Male	61 (55%)
Not specified	1 (1%)
Age at onset (days)	16 (1-86)

* Median (Ranges)

3.3 Clinical Presentation and Diagnosis

The common presenting signs were related to the respiratory system. The three most common presenting symptoms were an increase in the ventilatory requirements, temperature instability and abdominal distension (Table 3.2). Most of the patients were diagnosed as having candidemia as there was no specific clinical diagnosis given. These patients had clinical signs and a positive *Candida* culture. The common clinical diagnosis was NEC (35%). This is a primary diagnosis. The patients presented with NEC and subsequently developed fungal sepsis.

Table 3.2 Presenting Signs and Clinical Diagnosis in Patients with Fungal Sepsis

Signs	n (%)
General (n=49)	
Temperature instability	42 (22)
Lethargy	5 (2.6)
Gastrointestinal tract (GIT) (n=42)	
Abdominal distension	22 (11)
Nasogastric aspirates	14 (7)
Vomiting	6 (3)
Respiratory (n=84)	
Apnoeas	21 (11)
Respiratory distress and/or desaturating	63 (33)
Metabolic (n=18)	
Hyperglycaemia	13 (6.6)
Hypoglycaemia	5 (2.6)
Central nervous system (CNS) (n=2)	
Seizures	2 (1)
Diagnosis (n=111)	
Candidemia	54 (49)
Necrotizing Enterocolitis	39 (35)
Nosocomial Pneumonia	14 (13)
Meningitis	7 (6)

3.4 Laboratory Findings

Most patients (98%) had a full blood count (FBC) and CRP done as part of the work-up at the time of sepsis. The majority (82%) of patients had a normal white cell count (WCC) with only 7(6%) having leucopenia (WCC $<5 \times 10^9$/l) and 13 (12%) having a leucocytosis (WCC $>25x10^9$/l) (Table 3.3). Fifty-six percent of patients had a thrombocytopenia (platelet count $<150x10^9$/l). Seventy-three percent of patients had an abnormal CRP with 66% having a CRP of > 20mg/l.

Table 3.3 Initial and Repeat Laboratory Findings (FBC and CRP) Among Infants with Fungal Sepsis

	Initial	Repeat
	n=109	n = 99
White cell count*	13.3 (1.5-57.1)	12.8(2.6-43.5)
Leucopenia (< 5 x 10^9/l)	7 (6%)	10 (10%)
Normal (5-25 x 10^9/l)	89 (82%)	76 (77%)
Leucocytosis (>25 x 10^9/l)	13 (12%)	13 (13%)
	n=109	n = 99
Platelet count*	123 (5-755)	92(4-721)
Thrombocytopenia (<150x 10^9/l)	61 (56%)	62 (63%)
Normal count (150 – 450 x 10^9/l)	41 (38%)	31 (31%)
Thrombocytosis (>450 x 10^9/l)	7 (6%)	6 (6%)
	n=107	n = 88
C-reactive protein*	45 (1-542)	30.5(1-209)
Normal <10 mg/l	30 (27%)	18 (20.5%)
Borderline (10-20 mg/l)	8 (7%)	18 (20.5%)
Increased >20 mg/l	72 (66%)	52 (59%)

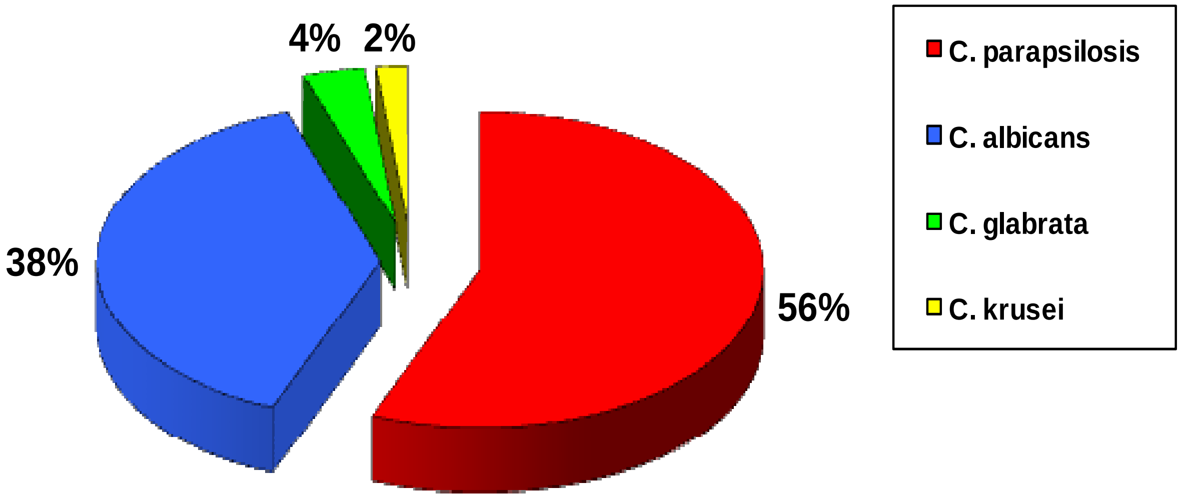
* Median (Ranges)

Ninety-nine (89%) of the 111 patients had a repeat FBC and 88 (79%) patients had a repeat CRP. Repeat FBC and CRP were requested at the discretion of the clinicians and are usually done when there is an abnormal count or if the patient has not improved clinically. The median number of days for both the FBC and CRP to be repeated was 3 days. Comparing the first FBC and the repeat FBC the number of patients with an abnormal WCC increased from 18% to 23% and the number of those with thrombocytopenia increased from 56% to 63% with the median platelet count decreasing from 123x10^9/l to 92x10^9/l. The number of patients with a high CRP increased from 73% to 79%. Overall there was a trend towards a decrease in platelet count and WCC, and an increase in the CRP.

3.5 Fungal Isolates

The common isolates identified among neonates with fungal sepsis were *Candida parapsilosis* (63) and *Candida albicans* (43) (Figure 5.1). Only 6 were caused by other species, 4 by *C. glabrata* and 2 by *C. krusei*. One patient had isolated two fungal species during the same episode of fungal sepsis that is a *C. albicans* and *C. glabrata*. The organisms were not cultured from the same blood specimen but cultured within 10 days of each other. The patient was treated with a combination of amphotericin B and fluconazole and survived. The combination therapy was used as *C. albicans* was repeatedly isolated from 3 cultures done at different times.

Figure 5.1 Fungal species causing sepsis in neonates



3.6 Susceptibilities

Among the commonly isolated species susceptibilities were available for 25 of the 43 (58%). *C. albicans* isolates for both amphotericin and fluconazole and 41 of the 63 (63%) *C. parapsilosis* isolates were available for amphotericin B and 40 of the 63 (63%) for fluconazole (Table 3.4). One of the *C. parapsilosis* isolates did not have susceptibilities documented for fluconazole. Among the *C. albicans* species with susceptibility results available 100% were sensitive to amphotericin B and 96% sensitive to fluconazole. Whereas for *C. parapsilosis* the susceptibility patterns among those with available susceptibility results were 93% for amphotericin B and 58% for fluconazole.

Table 3.4 Susceptibilities of Fungal Species Isolates to Antifungals

Organism (n)	Amphotericin B	Fluconazole
C. albicans (25)		
Susceptible	25(100%)	24 (96%)
Intermediate	0 (0%)	0 (0%)
Resistant	0 (0%)	1 (4%)
C. parapsilosis (41)		
Susceptible	38(93%)	23 (58%)
Intermediate	2 (5%)	9 (23%)
Resistant	1 (2%)	8 (20%)
C. glabrata (3)		
Susceptible	1 (33%)	3 (100%)
Intermediate	2 (67%)	0 (0%)
Resistant	0 (0%)	0 (0%)

3.7 Mortality

Among the 111 patients that were reviewed 27 died before hospital discharge giving an all-cause mortality rate of 24% for patients diagnosed with *Candida* (Table 3.5). Twenty-one patients died within 14 days of being diagnosed with *Candida*; giving a case fatality rate of 19% (Table 3.6). Case fatality rates for *C. albicans* and *C. parapsilosis* were high at 23% and 16% respectively. Only 1 patient among the 6 patients who were infected by *C. glabrata* and *C. krusei* demised.

Table 3.5 All –Cause Mortality of Infants with Fungal Sepsis according to Species

Organism	Number	All cause mortality	All-cause mortality
C. albicans	43	13	30%
C. parapsilosis	63	13	20%
C. glabrata	4	0	0%
C. krusei	2	1	50%
Total	112*	27	24%

* one patient had cultured 2 organisms

Table 3.6 Mortality of Infants with Fungal Sepsis

Organism	Number	*Candida* - related mortality	Case fatality rates
C. albicans	43	10	23%
C. parapsilosis	63	10	16%
C. glabrata	4	0	0%
C. krusei	2	1	50%
Total	112*	21	19%

* one patient had cultured 2 organisms

3.8 Comparing Survivors and Non-survivors in the Fungal Group

Table 3.7 is a comparison between the characteristics of the survivors and non-survivors in the fungal group for the deaths related to *Candida* sepsis. Comparing the survivors and non-survivors, the only difference noted was that the non-survivors were older (18 days vs 15 days, p=0.04) and had lower platelet counts ($39x10^9$/l vs $151x10^9$/l, p=0.007) than the survivors at the time of onset of sepsis. There were no significant differences in the birthweight, gestational age, gender and mode of delivery and maternal HIV status identified between the two groups.

Table 3.7 Comparison of the Characteristics Between the Survivors and Non-Survivors within the Fungal Sepsis Group for *Candida*-related mortality

	Survivors n=81	Non-survivors n=21	p-value
Birth Weight (g)*	1300 (660- 3300)	1145 (820-3200)	0.7
Gestational age (weeks) *	30 (23 – 39)	29 (26- 40)	0.6
Booked			0.104
Yes	69	14	
No	9	5	
Unknown	3	2	
Gender			0.245
Female	38	7	
Male	42	14	
Ambiguous	1		
Mode of Delivery			0.492
Vaginal	49	11	
Abdominal	27	5	
Maternal HIV status			0.87
Positive	25	6	
Negative	47	7	
Unknown	9	1	
Age at onset (days)*	15 (1 – 60)	18 (2 -86)	0.04
Initial	n=80	n=21	
WCC X 10^9/l*	12.45 (1.50-57.1)	13.5 (3.5-37.8)	p=0.990
Platelet count X10^9/l*	151.50 (5-755)	39 (5 – 422)	p=0.007
CRP mg/l*	37.2 (1- 542)	49 (2.2 -130	p=0.821
Repeat	n=73	n=18	
WCC X 10^9/l*	11.7 (2.69-37.4)	13.3 (4.4- 36.7)	p=0.423
Platelet count X10^9/l*	132.0 (9.0-721)	28 (4.0-331)	p=0.002
	n=14	n=66	
CRP mg/l*	23.3 (1.00-209)	79.4 (4.0 – 200.2)	p=0.013

*Median (Ranges)

3.9 Comparison Between the *C. albicans* Group and the *C. parapsilosis* Group

In comparing the infants who were infected by *C. albicans* to those with *C. parapsilosis*, the only difference was that those that were infected with *C. parapsilosis* were smaller (p=0.022) and had a lower gestational age (p=0.002) than those infected with *C. albicans* (Table 3.8).

Table 3.8 Characteristics of Patients in the *C. albicans* group compared to the *C. parapsilosis* group.

Characteristic	*C. albicans* n= 43	*C. parapsilosis* n= 63	p-value
Birthweight (g)*	1594.65 (850-3200)	1335.80 (660-3300)	0.022
Gestational age (weeks)*	32.20 (26.0-40.0)	30.04 (23.0-39.0)	0.002
Booking status			0.848
Yes	35	50	
No	7	9	
Unknown	1	4	
HIV status			0.119
Positive	15	16	
Negative	18	39	
Unknown	10	7	
Mode of delivery			0.136
Vaginal	29	23	
Abdominal	10	34	
Gender			0.272
Male	21	37	
Female	22	25	
Ambiguous		1	
Apgar			
1 minute*	6 (1-10)	6 (1-9)	0.962
5 minute *	8 (4-10)	7 (4-10)	0.443
Age at onset (days)*	20 (2-86)	18 (1-66)	0.61
Death			0.316
Yes	10	10	
No	29	47	

*Median (Ranges)

Table 3.9 Comparison of Initial and Repeat Blood Results Between the *C. albicans* and the *C. parapsilosis* Group

	C. albicans	*C. parapsilosis*	p-value
Initial	n=41	n=63	
WCC x10^9/l*	13.0 (1.50-30.9)	14.8 (3.5-37.8)	0.61
	n=41	n=63	
Platelet count x10^9/l*	136.8 (5.0-422.0)	179.7 (13.0-755.0)	0.162
	n=43	n=62	
CRP mg/l	63.7 (2.0-191.0)	53.5 (1.0-542.0)	0.463
Repeat			
WCC x10^9/l*	n=38	n=56	
	15.8 (8.3-22.1)	13.2 (8.2-15.6)	0.143
	n=38	n=56	
Platelet count x10^9/l*	147.9 (27.0-210.0)	144.2 (32.0-232.0)	0.912
	n=29	n=54	
CRP mg/l	62.2 (18.5-101.2)	40.9 (11.0-49.0)	0.031

*Median (Ranges)

There was no organism specific difference between the initial and repeat FBC with respect to the white cell count and platelets. However, there was a significant difference in the repeat CRP between the two groups with the *C. albicans* group having a much higher CRP count (p=0.031) (Table 3.9).

3.10 Risk Factors Associated with the Development of Fungal Sepsis

To determine the risk factors associated with development of fungal sepsis; a comparison was made between the study group and a convenient sample of infants infected with Gram-negative (GN) organisms. There was a significant difference in birthweight, gestational age and day of life of onset of sepsis between the two groups. The fungal sepsis group weighed less (p=0.002), and had a lower gestational age (p=0.03) but a later day of onset of sepsis (p=<0.01) and had lower Apgar scores at 1 min (p=0.044) and 5 min (p=0.048) when compared to the GN group (Table 3.10).

Table 3.10 Comparison of Characteristics of Infants with Fungal Sepsis to those with Bacterial Sepsis.

Characteristic	Fungal n=111	Bacterial n=112	p-value
Birthweight *	1280 9660-330)	1480 (780-3980)	0.002
Gestational Age*	30 (23 - 40)	32(25-42)	0.003
Day of life onset*	16 (1-86)	8 (1-90)	<0.001
Apgar			
1 minute*	7 (1-10)	8 (1-10)	0.044
5 minutes*	8 (4-10)	9 (3-10)	0.048
Maternal HIV status	n = 93	n=104	0.156
Positive	31(33%)	46 (44%)	
Negative	62 (67%)	58 (56%)	
Gender			0.303
Female	49	44	
Male	61	75	
Mode of delivery			0.677
Vaginal	66	82	
Abdominal	35	37	
Deaths related to sepsis	21 (19%)	28 (23%)	0.670

*Median (Ranges)

In comparing the laboratory results the WCC was significantly lower (p<0.001) in the bacterial group in both the initial and repeat blood results. The platelet count and CRP were similar between the two groups at the time of the sepsis work-up, however, on the repeat FBC; the platelet count was significantly lower (p=0.013) and the CRP (p=0.013) was significantly higher in the bacterial group. There was no statistically significant difference in mortality rate due to sepsis between the two groups (Table 3.11).

Table 3.11 Comparison of Initial and Repeat Laboratory Parameters Between Infants with Fungal and Bacterial Sepsis

	Fungal	Bacterial	p- value
Initial blood results			
	n = 109	n = 120	
White cell count*	13.3 (1.5 -57.1)	8.8(0.8-33.3)	<0.001
Leucopaenia (<5x10^9/l)	7 (6%)	32 (27%)	
Normal WCC (5–25x10^9/l)	89 (82%)	85 (71%)	
Leucocytosis (>25x10^9/l))	13 (12%)	4 (3%)	
	n = 109	n = 120	
Platelet count*	122 (5-755)	97 (5-510)	0.181
Thrombocytopaenia (<150x10^9/l)	61 (56%)	78 (65%)	
Normal (150–450x10^9/l)	41 (38%)	41 (34%)	
Thrombocytosis (>450x10^9/l)	7 (6%)	1 (1%)	
	n = 107	n = 100	
C-reactive protein*	43(1-542)	33.5 (1-314)	0.641
Normal (<10 mg/l)	30 (27%)	27 (27%)	
Borderline (10-20 mg/l)	8 (7%)	14 (14%)	
Increased (>20 mg/l)	72 (66%)	59 (59%)	
Repeat blood results			
	n = 99	n = 73	
White cell count*	12.8(2.6-43.5)	8.6 (1.19-31.5)	<0.001
Leucopaenia (<5 x 10^9/l)	10 (10%)	17 (23%)	
Normal WCC (5 – 25 x 10^9/l)	76 (77%)	49 (67%)	
Leucocytosis (>25 x 10^9/l))	13 (13%)	7 (10%)	
	n = 99	n = 72	
Platelet count*	92 (4-721)	45 (7 -393)	0.013
Thrombocytopaenia (<150x10^9/l)	62 (63%)	58 (81%)	
Normal (150– 450x10^9/l)	31 (31%)	14 (19%)	
Thrombocytosis (>450x10^9/l)	6 (6%)	0 (0%)	
	n = 88	n = 40	
C-reactive protein*	30.5(1-209)	72 (1-296)	0.013
Normal (<10 mg/l)	18 (20.5%)	5 (12.5%)	
Borderline (10-20 mg/l)	18 (20.5%)	5 (12.5%)	
Increased (>20 mg/l)	52 (59%)	30 (75%)	

*Median (Ranges)

There were more patients on antibiotics other than Penicillin G and Gentamicin (p=<0.001), who had central lines inserted (p=<0.001) and who were on TPN (p=<0.001) in the fungal sepsis group compared to the bacterial group (Table 3.12).

Table 3.12 Comparison of use of Antibiotics, Central lines, and TPN Between Infants with Fungal and Bacterial Sepsis

	Fungal n=111(%)	Bacterial n=122(%)	p-value
First line antibiotics			
Penicillin + Gentamicin	31(28)	60(49)	p=0.0018
Antibiotic other than first line	79 (71)	29 (24)	<0.001
Tazocin + amikacin	41(37)	13 (11)	
Meropenem + vancomycin	34 (31)	13 (11)	
Other	4 (3)	3 (2)	
Central lines	62 (56)	38 (31)	<0.001
Total parenteral nutrition	85 (76)	24 (20)	<0.001

CHAPTER 4

4. DISCUSSION

The advances made in newborn care have resulted in the premature neonate surviving longer and hence prone to interventions and nosocomial infections. Fungal sepsis is emerging as an important cause of sepsis in the neonatal population. One study found an eleven-fold increase in the rate of candidemia.[23] *Candida* species rank as the third and fourth most common organisms isolated in the neonatal units in the UK and in the USA respectively.[104] There are few studies on epidemiology of fungal sepsis from developing countries like South Africa. This study was undertaken to determine the epidemiology of *Candida* infections at the Chris Hani Baragwanath Hospital.

The main findings from this study were that 1. the common *Candida* species causing sepsis in neonates is *Candida parapsilosis*, 2. the common presenting signs in infants with fungal sepsis are apnoea, respiratory distress and non-specific signs like temperature instability and lethargy, 3. the common clinical diagnosis is necrotizing enterocolitis 4. thrombocytopaenia and a high CRP are found in majority of patients with fungal sepsis, 5. *Candida* sepsis is associated with high mortality, 6. Thrombocytopaenia and a high CRP are associated with a high mortality, 7. patients with lower birth weight/ gestational age, prolonged hospital stay, low Apgar scores, who have a central venous catheter, TPN and have been treated with Cephalosporins are at risk of having fungal sepsis, 8. In contrast to the common notion that thrombocytopaenia is a sign of fungal sepsis rather than bacterial sepsis, in this study the findings are that thrombocytopaenia is equally common to both bacterial and fungal infected neonates.

Studies reported a higher prevalence in the ELBW and VLBW group.[24,49] There was also an inverse relationship between gestational age and the acquisition of fungal sepsis. Benjamin et al reported that fungal sepsis is more common if the patient's gestational age was < 25 weeks.[10] In our study the median gestational age was 30 weeks and the birth weight 1500g, which is higher than that reported by Benjamin et al. This difference is accounted for by policies in the unit at the time of the study, where mechanical ventilation was not offered to neonates <1000g. Hence, patients did not live long enough to develop fungal sepsis. The smaller neonates being handled more due to nursing care and blood taking are more prone to infections. This coupled with the immature immune system and thin skin barrier make them more susceptible to nosocomial infections.[7] In addition our hospital is a very busy neonatal facility with an average of 3500 admissions per year during the study period. Inadequate hand hygiene techniques could have contributed to the increase in *C. parapsilosis* in our setting. The literature has identified the hands of health care workers as a source.[27,32,35] The median age of onset is 16 days. Saiman et al reported a median age of onset of 14 days for fungal colonisation, and another study documented a mean age of onset for fungal sepsis that ranged from 15 – 33 days similar findings to our study.[7,35]

Central venous catheters, TPN and third generation cephalosporins have been identified as risk factors for the development of fungal sepsis in this study. These findings are similar to other studies. Saiman et al identified similar risk factors in addition to the number of days the CVC was left in-situ, the use of H2 – blockers, and whether the patient was ventilated or not.[35] Colonisation was also a risk factor identified as this would increase the likelihood to develop fungal sepsis.[62,84,112,113] Factors that Chow et al identified that selected for NAC organisms were the receipt of fluconazole, the insertion of CVCs and the use of antimicrobials.[40] Abi Said et al also reported an increase in *C. parapsilosis* which was associated with the use of the use of TPN and CVCs.[22]

Candida parapsilosis was the commonest organism isolated. There were no significant differences in the risk factors between the infants who infected with *C. parapsilosis* compared to the infant infected with *C. albicans*. Some studies have reported *C. albicans* as the common organism.[35,51,52,80,114] The patients with *C. parapsilosis* were smaller and had a lower gestational age than the patients with *C. albicans*. This is similar to the findings by Shoa et al.[100]

Thrombocytopenia occurs in 20-50% of all neonates admitted to the NICU and the incidence is 50% among preterm infants.[115] Thrombocytopenia is used as a non-specific marker for sepsis in the neonate.[116] This study showed that patients infected with GN organisms had lower platelet counts than patients with fungal sepsis. Guida et al reported similar findings but used a cut-off of <100000x10^{9}/l to define thrombocytopenia.[62] We used a platelet count of <150000x10^{9}/l in the definition of thrombocytopenia. Bhat et al found a lower platelet count in their GN group of patients.[115] They also identified that K.pneumoniae was the organism that caused the most effect on the platelet count. This is due to a lipopolysaccharide component, Lipid A which amplifies the interaction between the IgG Fc receptor and the organism and this causes a further increase in platelet consumption. A similar mechanism has been proposed for fungal sepsis but has not been identified. Another study found no difference in the platelet counts between the GN and gram positive and fungal organisms.[117] However, this study did not look at the severity or duration of the thrombocytopenia. These may be important characteristics. Benjamin et al reported *Candida* organisms to be most associated with thrombocytopenia and formed part of his clinical predictive model.[10] The other factors that could cause thrombocytopenia such as maternal steroids, pre-eclampsia, maternal diabetes, maternal ITP were not assessed in this study.

All of the *C. albicans* isolates were sensitive to fluconazole and amphotericin B. The Sentry antimicrobial surveillance programme in the USA reported that 95% of the *C. albicans* isolates were sensitive to amphotericin B.[93] Whilst the TSARY surveillance programme in Taiwan reported an increase in resistance of all isolates to amphotericin B from 0.5% in 1999 to 2.5% in 2002 and a decrease in the resistance rate to fluconazole from 8.45 to 1.9%.[79] Yet there were only 3 of the 395 isolates of *C. albicans* resistant to amphotericin B in TSARY 2002.[106] Amongst the *C. parapsilosis* isolates 93% were susceptible to amphotericin B and 20% were documented to have resistance to fluconazole. This is consistent with the findings in the Artemis Disk antifungal surveillance programme were 20.3% of *C. parapsilosis* isolates from South Africa were resistant to fluconazole.[27] The region with the most susceptible isolates was found in Europe. Clerihew et al reported that all the *C. parapsilosis* to be sensitive to amphotericin B.[36] The *C. glabrata* isolates were all sensitive to fluconazole and two-thirds showed a susceptible dose-dependent (S-DD) susceptibility profile to amphotericin B. Many studies are reporting an emerging resistance of *C. glabrata* and *C. krusei* to both antifungals and the IDSA guidelines recommend a higher dose of these drugs when treating these two isolates.[63,102] Fluconazole prophylaxis selects for the isolation of these organisms hence, when treating patients with these two isolates an appropriate antifungal and dose should be commenced. During the period at which the data from this study was collected, fluconazole was not prescribed for prophylaxis. The one *C. krusei* isolate in this study was sensitive to amphotericin B and had intermediate resistance to fluconazole. This is in contrast to studies where *C. krusei* is resistant to fluconazole and having decreased susceptibility to amphotericin B.[95,118] The significance of these findings is difficult to interpret as there were only two isolates of *C. krusei.*

Fungal sepsis has a mortality rate of 20 – 40% with the rate being higher in the ELBW (37 – 40%) as compared to the VLBW infant (32%).[47,51] *Candida albicans* had a higher mortality than *C. parapsilosis* in this study. This is similar to findings reported by Kauffmann et al where mortality of *C. albicans* vs *C*. *parapsilosis*, was 44% vs 16% respectively. Stoll and Saiman et al had similar findings.[8,9,35,109] The higher mortality associated with *C. albicans* infection is due to the organism's virulence factors. It tends to adhere tighter to epithelial cells than *C. parapsilosis* and causes a more lethal clinical presentation.[31] Whilst *C. parapsilosis* is less virulent it is more difficult to eradicate and this compounds the morbidity related to this isolate. Other studies found no difference in mortality between the two organisms.[36] Thrombocytopenia is associated with death in patients with *Candida* sepsis.[26,115] It was reported by Bhat and colleagues that mortality was higher; 36% compared to 16% in patients with thrombocytopenia as compared to patients without thrombocytopenia. In this study a significant difference in platelet count was found in the repeat blood count between the survivors and non-survivors. Non-survivors had a much lower platelet count than the survivors. *Candida albicans* has emerged as the organism with the highest case fatality rate despite being the second commonest organism isolated. Fluconazole prophylaxis in a select group of patients would alter the prevalence of this organism and thereby decrease the overall mortality rate. A randomised control trial would need to be performed in the unit as detailed by Kauffmann as this may alter the epidemiology of fungal sepsis in the neonatal unit. We should then be aware that the prevalence of the NAC may increase and alter the susceptibility profiles of the organisms. The unit policy has subsequently changed where ventilation is offered to neonates < 1000g. This will influence the survival of neonates in our setting and influence the epidemiology of candidemia.

The study identified the subset of patients that would develop fungal infections. In our setting if a VLBW had risk factors such as having had central lines, been on TPN and third generation cephalosporins and had abdominal surgery; we would empirically start antifungal treatment. Thrombocytopenia is not a pathognomonic feature as patients with GN sepsis would also present with a low platelet count[62]. It would be more important to look at the persistence of the thrombocytopenia and to compare the nadir of the low platelet count. Thrombocytopenia is commonly used to identify patients with fungal sepsis but from this study thrombocytopenia was not exclusively associated with fungal sepsis. These patients should be treated with antibacterials first and in cases where patients are not responding antifungal treatment should be considered.

At present the choice of antifungals need not be altered as the resistance of the commoner isolates is not posing a serious problem. Trends need to be followed via a surveillance programme so as to identify emerging resistance as is seen in the rest of the world. This would then have an impact on future prescribing practices.

CHAPTER 5

CONCLUSION

In conclusion the VLBW and smaller infant are more prone to developing fungal sepsis. A lower platelet count is associated with an increased mortality. Having risk factors like the use of TPN, a CVC in-situ and a third generation cephalosporin places the VLBW infant at higher risk for fungal sepsis. Great benefit will be gained if a surveillance programme is undertaken. Fungal sepsis is a major cause of infection and its associated mortality in neonates born or admitted at Chris Hani Baragwanath Hospital. Its epidemiology is similar to what has been reported in other studies. Clinicians need to monitor development of resistance to fluconazole as some *Candida* species like *C. parapsilosis* have been found to be resistant to fluconazole.

CHAPTER 6

LIMITATIONS

This was a retrospective study hence many patient records were not located. The data collection was biased as to what had been written in the patient files. A convenient sample of patients with GN sepsis was used as a comparison to identify risk factors. Hence, they were not matched controls. Furthermore, the GN group was collected over a one year period and the fungal group over a three year period. It would have been ideal to get controls from patients who were not infected.

With regard to the laboratory parameters, not all the patients had repeat laboratory markers performed. Had this been a prospective study we would have had increased numbers of repeat specimens and this could alter the trends observed. No other causes for the thrombocytopenia were looked at eg. maternal steroids and preeclampsia or the possibility of a congenital infection. The other pitfall in this study included that we did not look at the duration and nadir of the thrombocytopenia and the change in platelet count from baseline (i.e. before the onset of sepsis).

Risk factors that contribute to the development of fungal sepsis are many. We reported on three. We had not taken into account other risk factors such as colonisation rates among the VLBW infants, whether the infant had been ventilated or not, if there was any abdominal surgery and if the neonate had received any steroids or H2 blockers. The number of days that the CVC had been in-situ is another important factor and if the CVC was removed and how this affected clearance rates of the fungal isolate.

Susceptibilities for all the organisms were not routinely done by the laboratory at the time, therefore, only two-thirds of the isolates had susceptibilities documented. Hence, the susceptibility profile may be a bit skewed.

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APPENDIX A

Candidates Surname: Nakwa	First Name: Firdose Lamboy	Student No.: 9102664/T
Current Qualifications: MBBCh(Wits); FC Paeds(SA)		
Tel: 011 933 8000	Cell: 072 414 0622	E-mail: firdosen@hotmail.com Fax: 933 9516
Degree for which protocol is submitted: MMed (Paeds) Code: MMJ00		
Part Time Or Full Time: Full Time		
First Registered For This Degree	Term: 01 January	Year:2001
Department: Paediatrics		
Title of proposed research: FUNGAEMIA IN THE NEONATAL UNIT AT CHRIS HANI BARAGWANATH HOSPITAL: RISK FACTORS, AETIOLOGY, SUSCEPTIBILITY TO ANTIFUNGALS AND OUTCOME.		
Candidate's Signature: Nakwa	Date: 07/09/2004	
Supervisor's Name: Dr. S. Velaphi		
Supervisor's Qualifications: MBChB, FCPaeds		
Supervisor's Department: Paediatrics, University of the Witwatersrand		
Co- Supervisor's Name:		
Co-Supervisor's Department:		
Co-supervisor's Address/Tel/E-Mail:		
Synopsis of Research: Fungi are important pathogens causing sepsis during the neonatal period. Invasive candidaemia is associated with substantial morbidity and mortality. Infants not treated with antifungals have a high mortality. In the Baragwanath neonatal unit, antifungal therapy is often started empirically if a patient with suspected neonatal sepsis has negative blood cultures and is not responding to antibiotic therapy. Therefore, it is important to know which candida species are prevalent in our unit, which antifungals they are susceptible to and their case-fatality rates. The objectives of the study will be the following: 1) to determine the incidence of fungal sepsis in the neonatal unit and the risk factors associated with the development of fungal sepsis by comparing them to those with bacterial sepsis; 2) to identify the different fungal species causing sepsis and their susceptibility to the antifungals used; 3) to determine the clinical features and laboratory parameters associated with the fungus; 4) to determine the case fatality rate of the different fungal species. It will be a retrospective study, reviewing records of patients with positive fungal and bacterial cultures from January 2000 to June 2004.		
Ethics Approved: YES	Ethics Clearance Number: M03-10-38	
Signature of Supervisor/s:		

FUNGAEMIA IN THE NEONATAL UNIT AT CHRIS HANI BARAGWANATH HOSPITAL: RISK FACTORS, AETIOLOGY, SUSCEPTIBILTY TO ANTIFUNGALS AND OUTCOMES.

PROTOCOL FOR A STUDY BY

FIRDOSE LAMBEY NAKWA

Student number: 9102664/T

Staff number: 00100041

Outline

FUNGAEMIA IN THE NEONATAL UNIT AT CHRIS HANI BARAGWANATH HOSPITAL: RISK FACTORS, AETIOLOGY, SUSCEPTIBILITY TO ANTIFUNGALS AND OUTCOME.

BACKGROUND

Fungi are important pathogens causing sepsis during the neonatal period. Premature and critically ill infants are more susceptible to developing fungal infections[1]. With increasing survival of very low birth weight infants, fungaemia contributes to an increase in mortality and morbidity[2,4,5] .

Sepsis due to fungi accounts for 2% to 9% of hospital acquired infections, an estimated 3 –5% of infants with a birth weight <1500g and 10% of infants with a birth weight <1000g[4]. The development of fungal sepsis is associated with certain risk factors. These include prematurity, prolonged hospital stay, prolonged use of intravascular catheters, prolonged use of hyperalimentation, especially intravenous fat emulsions, duration of systemic antibiotics, and the use of aminophylline[1,2,3]. The rate of candidaemia is inversely related to the gestational age[6] .

Among the fungi, the common genoma causing infections in neonates is candida. Candidaemia is the third most common cause of late-onset neonatal sepsis amongst patients admitted in neonatal intensive care units (NICU), with a crude mortality rate that varies between 15 – 50%[5] . There are 80 different species of Candida, 10 of which are clinically significant and have been implicated in human infections: C. albicans, C. parapsilosis, C.tropicalis, C.stellatoidea, C. krusei, C.guillermondii, C.pseudotropicalis, C. glabrata, C.lusitaniae, and C.rugosa. The common candida species isolated in neonates with sepsis are C. albicans, C. parapsilosis, and rarely, C. glabrata and C. tropicalis. Candida albicans accounts for the majority (80-90%) of fungal infections in neonates[1,4,5] . Candida albicans and C. glabrata are normal commensals in the gut. Candida parapsilosis is always a pathogen and has been isolated from the hands of health care workers; outbreaks have been reported with the use of total parenteral solutions and intralipid solutions, or contaminated pressure monitoring devices[7]. Recent studies have shown a shift from C.albicans infection to C.parapsilosis as the more prevalent species. However the mortality rate for C.albicans is still significantly higher[7,9].

Candida sepsis is difficult to diagnose. Only 50 – 80% of infected patients have positive blood cultures[8]. Due to its slow growth, the blood culture results are often delayed, resulting in delay in starting antifungal treatment. This delay may be associated with high mortality and morbidity, therefore identification of infants who are most likely infected with candida and initiation of early treatment may help to reduce mortality[8]. Candida albicans case fatality rate is 26% compared to a 4% case fatality rate of C.parapsilosis[9].

Clinical features of fungal sepsis are insidious and non – specific.It is therefore important to identify factors that are associated with increased risk of developing fungal sepsis. Laboratory parameters that have been found to be helpful in making a diagnosis of fungaemia are neutropaenia and/or thrombocytopaenia. Candida albicans has a greater increase in immature:mature neutrophil count as compared to C.parapsilosis. Thrombocytopaenia is a symptom rather than a cause of candidaemia. Studies have reported a greater decline in platelet count in C.albicans candidaemia compared to C.parapsilosis[8]. A number of factors as described by Benjamin et al have reported thrombocytopaenia, gestational age and the use of a third generation cephalosporin or carbepenem as being positive predictors of subsequent candidaemia[10].

The cornerstone of treatment is antifungals. However, a limited number of antifungals are available, their use being limited by their safety profile and efficacy. Most are fungistatic and achieve a minimum inhibitory concentration of 80%[13]. Amphotericin B, the common antifungal used, can cause renal, liver and other infusion toxicities. Lipid formulations of Amphotericin B can reduce this significantly, but their use is limited by cost[1,13]. Fluconazole, another agent, has good oral absorption but a narrow spectrum of activity, leading to the emergence of resistance. This anti-fungal has been shown to be as effective as Amphotericin B, but with less side effects[1,12,13,14,15].

Susceptibility to antifungals is species dependent. Whilst most centers use amphotericin B as empirical treatment, it should be borne in mind that resistance does occur. Primary resistance occurs in C.glabrata, C.guillermondii, C.krusei, and C.lusitaniae[15] . These organisms have a high propensity to possess or develop resistance to amphotericin B. C.albicans, C.guillermondii, C.lusitaniae have been described as developing secondary resistance whilst on therapy. With the escalating HIV epidemic, there has been an increase in isolates of C.albicans that are resistant to fluconazole[14,15]. Fortunately, the introduction of HAART has seen the decline of these azole-resistant strains[15,16]. Most neonatologists would use amphotericin B as empirical therapy for systemic fungal infection. It is wise to opt for susceptibility testing to institute the correct treatment. Fluconazole and amphotericin B combinations are a controversial issue, and their use is not recommended[15].

Chris Hani Baragwanath neonatal unit is a tertiary center, and the major referral for the south, south – western areas, including secondary hospitals and maternal obstetric units. With the ever changing socio-political environment and the influx of a rural population to urban areas, the patient numbers are on the increase, yet the bed status and staffing remains the same. A study undertaken by PA Cooper et al at Baragwanath hospital in1996 reported an increase in survival of the low birth weight infant due to the advances made in the care of the neonate. These advances include mechanical ventilation, artificial surfactant and total parenteral nutrition. Interestingly enough, the bed status remains the same as that reported in 1996, 12 NICU beds and 25-30 high care beds[17]. Staffing remains unchanged as well. However, the number of obstetric deliveries has increased over the years. The increased scientific advances promote the survival of the low birth weight infant, facilitating a prolonged stay in the neonatal unit. With the escalating HIV epidemic, a great proportion of mothers are HIV positive, thus contributing to the increased incidence of low birth weight infants. This causes overcrowding and makes infection control difficult. It is our impression that there is an increase in the number of infants presenting with sepsis, including fungal sepsis. Therefore, it is important to determine factors that are associated with the development

OBJECTIVES

1) To determine the incidence of fungal sepsis in the neonatal unit.
2) To determine the risk factors associated with the development of fungal sepsis by comparing them to those with Gram-negative bacterial sepsis.
3) To identify the different fungal species causing sepsis and their susceptibility to the antifungals used.
4) To determine the clinical features and laboratory parameters associated with the fungus.
5) To determine the mortality and case fatality rate of the different fungal species.

STUDY DESIGN

The study will take the form of a retrospective review of patient records. The patients will be identified by positive fungal cultures as per microbiological database in the neonatal unit at the Chris Hani Baragwanath hospital. This will be done over a two and a half year time period; January 2002 – June 2004. Control groups will be identified by positive Gram negative bacterial cultures over the same time period. Based on our microbiology database the estimated sample size is 228 cases of fungal sepsis and 500 cases of gram negative organisms over this two and a half year period.

ETHICS

Ethics approval has been sought and granted in October 2003 by the University ethics committee.

DATA COLLECTION

This will be done as per data sheet (see attached form) where a number of parameters will be studied as highlighted in the objectives above.

DATA ANALYSIS

Appropriate statistical analysis methods will be employed. A descriptive analysis of infants infected with fungal sepsis will follow. A comparison between cases and controls will be done using a student *t* test for continuous variables, a chi2 test for dichotomous variables.

IMPLICATIONS

The commonest fungal species affecting the infants in the neonatal unit at Baragwanath hospital will be identified including its susceptibility to antifungal therapy. This will allow the unit to commence empirical antifungal therapy earlier according to the susceptibility profile. It will also allow earlier identification of susceptible infants based on clinical and laboratory parameters. Risk factors associated with morbidity and mortality will be highlighted, hence patients can be timeously identified and fatalities prevented.

BUDGET

This being a retrospective study funding would cover stationary and photocopying. The investigator will finance this.

TIMELINES

The aim would be to collect data as soon as the protocol is approved, starting October 2004. During November analysis of the data would be undertaken. Writing up of the analyzed data will be done in December 2004.

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APPENDIX B

DATA COLLECTION SHEET: FUNGAL SEPSIS

1. **Infant details**

ID Number: …………………………………………………..

Culture date: ……/……/……. ; Episode (No): ……. ; Ward: ………. ; Admission date: ……./……./…….

Patient's Hosp No: …………………………………………………… ; Date of Birth: ……./……./…….

Place of Birth: ………… (1=Bara, 2=Clinic, 3=Home, 4=Other Hosp)

Birthwt: ………. ; GA: ………. ; (Based o Obstets/Paeds): ……… ; Sex: …….. ; Race: ……………….. ; Apgar: 1'…../5'……..

Mode of delivery: C/S, NVD, Forceps, Vacuum

Maternal HIV status: Pos / Neg / Unknown

---

2. **Presentation**

Date of onset of symptoms: ……./……./……. ; Day of life at onset: ……………………………..

Symptoms (Reason for sepsis work up): ………..

Diagnosis: ……………………………………………………………………………………

Antibiotics started on (date): …………………………………………………………..

Previous antibiot. Stopped: ……./……./…….

Date previous antibiot. Stopped: ……./……./…….

Ventilated: Yes / No; If Yes, Date intubated: ……./……./……. ; Date extubated: ……./……./…….

On Oxygen Headbox / Cannulae: Yes / No

Central line: Yes / No; Type: UAC/UVC/Other; Date inserted: ……./……./……. ; Date removed: ……./……./…….

Peripheral line: Yes / No

TPN: Yes / No, If yes, Date started, ……./……./……. ' Intralipids: Yes / No

FBC done with sepsis w/u: Wbc: …….   Hb: ……. Plts: ……. (Date of FBC: ……./……./…….)

Diff Count: Neutr…….; Lymph: ……..; Mono: …….; Eos: ……; Baso: ……. Others: ……………..

Smear results:
……

CPR done with sepsis w/u: ……….

CSF done with or around time of sepsis w/u: Yes / No; If Yes , date CSF done: ……./……./…….

CSF Results: Polys: …………; Lymphs: ……….; Rbcs: ……….; Protein: ……….; Gluc: ……….; Cl: ……….

FBC immed. After sepsis w/u: Wbc: ………. Hb: ………. Plts: ………. (Date of FBC: ……../……../……..

Diff Count: Neutr ……….; Lymph:……….; Mono: ……….; Eos: ………. Baso:………. Others: ……….

Smear results:
……

Repeat CRP done: ………………….

CSF Repeated: Yes / No; If Yes, date CSF repeated: ………/………./……….

CSF Results: Polys: ……….; Lymphs: ……….; Rbcs: ……….; Protein: ……….; Gluc:……….; Cl:……….

3. **Organism and susceptibilities**

Organism 1: …………………………………………………………………………….;

Culture Site: Bld / CSF

If fungal, Sensitivity: Fluconazole – R/S; Amphotericin B-R/S

Organism 2: …………………………………………………………………………….;

Culture Site: Bld / CSF

If fungal, Sensitivity: Fluconazole- R/S; Amphotericin B- R/S

Culture repeated: Yes / No; Date culture repeated: ………../………/………; Repeat culture: Positive / Negative

If culture positive, Organism: ………………………………………………..;

If fungal, Sensitivity: Fluconazole – R/S; Amphotericin B- R/S

4. **Outcome**

Died: Yes / No; If died, date of death: ………/………./……….

Cause of death: …….

Postmortem: Yes / No; If done, diagnosis after postmortem:……………………………………………………..

Other Comments:…….

APPENDIX C

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

COMMITTEE FOR RESEARCH ON HUMAN SUBJECTS (MEDICAL)
Ref: R14/49 Velaphi et al

CLEARANCE CERTIFICATE **PROTOCOL NUMBER** M03-10-38

PROJECT Fungaemia in Neonates Admitted in the Neonatal Unit at CH Baragwanath Hospital: Aetiology, Antifungal Susceptibilities and Outcome

INVESTIGATORS Drs S et al Velaphi et al

DEPARTMENT School of Clinical Medicine, CH Baragwanath Hospital

DATE CONSIDERED 03-10-31

DECISION OF THE COMMITTEE Approved unconditionally

Unless otherwise specified the ethical clearance is valid for 5 years but may be renewed upon application
This ethical clearance will expire on 1 January 2008.

DATE 03-11-02 CHAIRMAN...(Professor P E Cleaton-Jones)

* Guidelines for written "informed consent" attached where applicable.

c c Supervisor:

Dept of ,

Works2\lain0015\HumEth97.wdb\M 03-10-38

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DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and ONE COPY returned to the Secretary at Room 10001, 10th Floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. I agree to a completion of a yearly progress form. I/we agree to inform the Committee once the study is completed.

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Dr Firdose Nakwa

CLEARANCE CERTIFICATE M110848

PROJECT Fungaemia in the Neonatal Unit at Chris Hani Baragwanath Academic Hospital: Risk Factors, Aetiology, Susceptibility to Antifungals and

Outcome (Previously M031031 S Velaphi et al)

INVESTIGATORS Dr Firdose Nakwa.

DEPARTMENT Department of Paediatrics/Neonatology

DATE CONSIDERED 26/08/2011

DECISION OF THE COMMITTEE* Renewal Approved

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 26/08/2011 **CHAIRPERSON** ....
pp. (Professor ~~PE Cleaton-Jones~~)

UNIVERSITY OF THE WITWATERSRAND
PROF AJ WOODIWISS
Co-Chair
2011-09-27
HREC (Medical)
JOHANNESBURG

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor : Prof Sithembiso Velaphi

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and ONE COPY returned to the Secretary at Room 10004, 10th floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...

UNIVERSITY OF THE WITWATERSRAND
PROF AJ WOODIWISS
Co-Chair
2011-09-27
HREC (Medical)
JOHANNESBURG