THE PREVALENCE OF BACTERAEMIA IN NEUTROPENIC PAEDIATRIC CANCER PATIENTS ON CHEMOTHERAPY AT THE UNIVERSITY TEACHING HOSPITAL IN LUSAKA, ZAMBIA.

BY

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A dissertation submitted to the University of Zambia in partial fulfilment of the requirements of the degree of Master of Medicine in Paediatrics and Child Health

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DECLARATION

I declare that this dissertation represents my own work and that it has not been submitted for a degree, diploma or other qualification at this or any other university.

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ABSTRACT

Title: The Prevalence of Bacteraemia in Neutropenic Paediatric Cancer Patients on Chemotherapy at the University Teaching Hospital in Lusaka, Zambia.

Background: Bacteraemia in neutropenic paediatric cancer patients can lead to high morbidity and mortality if not treated properly. The prevalence of bacteraemia and antibiotic sensitivities are liable to change according to region and time. The study investigated the pattern of bacteraemia and antibiotic sensitivity patterns in neutropenic paediatric cancer patients at the University Teaching Hospital in Lusaka, Zambia.

Methods: A descriptive and analytical cross-sectional study was done at the Paediatric Oncology Ward of the University Teaching Hospital in Lusaka, Zambia. We evaluated 100 neutropenic episodes in 53 paediatric cancer patients with suspected neutropenic sepsis. These were enrolled following a consent procedure. Information was obtained from an interviewer administered questionnaire and the attending physician’s case notes. One millilitre of blood was then drawn for blood culture and sensitivity testing, and another one millilitre for full blood count. Information was entered using Epi info version 7 and analysed using SPSS version 2.1 for windows.

Results: The prevalence of bacteraemia in neutropenic paediatric cancer patients in this study was five per cent (5%). Four patients with positive blood cultures were in a critical condition (p value of 0.02). Gram negative organisms were isolated more often than gram positive organisms (80 per cent versus 20 per cent). Gram negative bacterial isolates were Enterobacter agglomerans (20%), Escherichia coli (20%), Pseudomonas aeruginosa (20%), and Klebsiella pneumoniae (20%). One gram positive bacterium
isolated was *Staphylococcus aureus* (20%). All gram negative isolates were from in-patients while the gram positive bacterium isolated was from an outpatient. In antibiotic sensitivity tests, two of four isolates (50%) of gram negative bacteria were sensitive to ciprofloxacin, two of four isolates (50%) were sensitive to gentamicin, and zero of four isolates were sensitive to cefotaxime. *Escherichia coli* isolate was resistant to ciprofloxacin. In the case of gram positive bacterium, *Staphylococcus aureus* was sensitive to chloramphenicol and clindamycin but resistant to penicillin and oxacillin.

**Conclusion:** Bacteraemia in paediatric cancer patients at the UTH in Lusaka mostly affects a young population of children (median age 6 years) in advanced stage of their disease. Commonest malignancies involved are leukaemias and lymphomas. Most patients are not infected with HIV. The major causative organisms of bacteraemia are gram negatives (80%) with high antimicrobial resistance to the commonly used antibiotics on the oncology ward. The choice of antibiotic in treating neutropenic paediatric cancer patients with suspected bacteraemia needs to take into account the prevalence of gram negative bacteraemia among in patients and antibiotic resistance of gram negative and gram positive bacteria.
DEDICATION

This study is dedicated to my father, Mr Newton Chingo, and my mother, Mrs Emily Chingo, for their dedication and sacrifice in ensuring that my sisters and I got the best education.

To my beloved husband, Mudukula Mukubi, my sons Choolwe and Lukamantano, for the support they gave me during the undertaking of this study.
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<th>Description</th>
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<tbody>
<tr>
<td>AIDS</td>
<td>Acquired Immune-Deficiency Syndrome</td>
</tr>
<tr>
<td>ALL</td>
<td>Acute Lymphoblastic Leukaemia</td>
</tr>
<tr>
<td>AML</td>
<td>Acute Myeloid Leukaemia</td>
</tr>
<tr>
<td>ANC</td>
<td>Absolute Neutrophil Count</td>
</tr>
<tr>
<td>ESMO</td>
<td>European Society for Medical Oncology</td>
</tr>
<tr>
<td>FBC</td>
<td>Full Blood Count</td>
</tr>
<tr>
<td>FN</td>
<td>Febrile Neutropenia</td>
</tr>
<tr>
<td>HIV</td>
<td>Human Immune-Deficiency Virus</td>
</tr>
<tr>
<td>HL</td>
<td>Hodgkin’s Lymphoma</td>
</tr>
<tr>
<td>MRC</td>
<td>Medical Research Council</td>
</tr>
<tr>
<td>NHL</td>
<td>Non-Hodgkin’s Lymphoma</td>
</tr>
<tr>
<td>NCI-CTCAE</td>
<td>National Cancer Institute-Common Terminology Criteria for Adverse Events</td>
</tr>
<tr>
<td>PS</td>
<td>Partially sensitive</td>
</tr>
<tr>
<td>RBC</td>
<td>Red Blood Cell</td>
</tr>
<tr>
<td>R</td>
<td>Resistant</td>
</tr>
<tr>
<td>S</td>
<td>Sensitive</td>
</tr>
<tr>
<td>UNZA</td>
<td>University of Zambia</td>
</tr>
<tr>
<td>UTH</td>
<td>University Teaching Hospital</td>
</tr>
<tr>
<td>WBC</td>
<td>White Blood Cell</td>
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DEFINITIONS

**Bacteraemia**: the presence of viable bacteria in the circulating blood.

**Critical condition**: a patient with unstable vital signs and not within normal limits.

**Fever**: a core body (rectal) temperature of $\geq 38.3^\circ C$ in children less than 24 months, or temperature $> 38^\circ C$ in those above two years with a change in the set point of the body.

**Hyperthermia**: a rapid rise in the body temperature of more than $38^\circ C$ which occurs without a change in the set point. There is excessive heat in the body due to failure in the mechanism of thermoregulation.

**Hypothermia**: body temperature below the normal range (36.5-37.5$^\circ C$) which could be mild (36-36.5$^\circ C$), moderate (32-35.9$^\circ C$), or severe (<32$^\circ C$) hypothermia.

**Sepsis**: the presence of pathogenic organisms or their toxins in the blood and tissues which trigger a systemic inflammatory response syndrome.

**Shock**: clinical syndrome of circulatory dysfunction resulting in inadequate delivery of oxygen and other nutrients to meet tissue metabolic demands.

**Tachycardia**: an abnormally rapid heart rate for age.

1-2 years > 150 beats/ minute, 2-5 years > 140 beats/ minute, above 5 years > 120 beats/ minute

**Tachypnoea**: a respiratory rate > 40 breaths/minute in children $\geq 1$ year old, > 30 breaths/ minute in children aged 2-5 years, > 25 breaths/ minute in children aged 5-12 years, and > 20 breaths/ minute in children above 12 years old.
CHAPTER 1

1.0 INTRODUCTION

Paediatric patients with haematological malignancies or solid tumours undergoing intensive cytotoxic chemotherapy or radiation therapy that cause granulocytopenia are at high risk of infectious complications. Chemotherapy, radiation therapy, or a combination of both interrupts bone marrow production of white blood cells (WBC), red blood cells (RBC), and platelets causing granulocytopenia, anaemia and thrombocytopenia, respectively. Granulocytopenia causes serious infections in this paediatric patient population and remains a leading cause of mortality (Penack et al., 2011).

Some paediatric cancer patients have a much higher risk of infection because of the cancer itself which, in addition to the cancer treatment, affects their bodies’ defence systems. Children at high risk of infection and mortality include those with acute lymphoblastic leukaemia (ALL), acute myeloid leukaemia (AML), and relapsed acute lymphoblastic leukaemia. Paediatric AML treatment related mortality ranges from 4 to 11% globally, with most of this mortality attributed to infection. Treatment related mortality of 9.6% in 1992-95 was reported for children treated in the United Kingdom according to the Medical Research Council (MRC-10), of which infection accounted for 65.9% of the deaths (Stevens et al., 1999).

Incidence of bacteraemia in febrile neutropenic patients is 10-27% worldwide (OudeNijhuis et al., 2002). Incidence of infections, according to Rahaila et al, (1998) occurring during the course of anti-cancer chemotherapy in children is 50%. In a study
done in Belgium by Klastersky et al (2007) on bacteraemia in febrile neutropenic cancer patients, the incidence of febrile neutropenia was 23%. In Malawi, treatment related mortality in 2000 was 26%, with 16.7% of the children presumed to be due to bacterial infection (Hesseling et al., 2005).

The relationship between fever, neutropenia and bacteraemia has been widely known for more than forty years. Certain characteristics of the neutropenic episode such as severity, course, duration and time of resolution determine vulnerability of patients and outcome when they develop an acute infection (Pizzo, 1999).

Over the past three decades, considerable changes have occurred in the types of bacteria causing infection in febrile patients with neutropenia with a shift toward gram positive coccal bacteraemia. Of concern is that anti-microbial resistant gram-positive organisms are becoming increasingly frequent in patients with neutropenia. On the other hand, fluoroquinolone resistant Escherichia coli are still being isolated from several cancer centres (Zinner, 1999).

However, bacteraemia is an underestimated health problem for most African societies (Berkley et al., 2005). Indeed there is a paucity of research on the epidemiology of bacteraemia in cancer patients in Zambia. In the study period, averages of 26-30 paediatric cancer patients were admitted per month at the oncology ward from different parts of the country. Based on observations by the physicians at UTH, suspected neutropenic sepsis contributed to increased morbidity and mortality. This study seeks to determine the prevalence of bacteraemia in neutropenic paediatric cancer patients at the University Teaching Hospital in Lusaka, Zambia.
1.1 Statement of the Problem

Neutropenia is common among children receiving cancer therapy. This results in neutropenic sepsis, (an oncological emergency) that contributes to increased morbidity and increased mortality if not treated early and properly.

Inappropriately treated patients according to Spanik et al (1998) have been known to have poorer outcomes. An appropriate regimen in the empiric therapy of bacteraemia associated with neutropenia in paediatric cancer patients must be individualised at each institution.

However, organisms causing sepsis in neutropenic cancer patients at the University Teaching Hospital are not known. As such, there is no evidence-based protocol in the oncology unit at the University Teaching Hospital in Lusaka. Therefore, no data is available in Zambia for recommendation on the empiric treatment of neutropenic sepsis in paediatric cancer patients based on antimicrobial susceptibility pattern.
1.2 Justification

Bacteraemia is an underestimated health problem for most African countries, as has been shown by Berkley et al (2005). To draw conclusions about therapy and prevention, additional knowledge of rates of resistance in various geographical settings is necessary. There is a knowledge gap on the prevailing pattern of bacterial infections and sensitivity patterns among neutropenic cancer patients. Because most deaths from bacteraemia illnesses occur very early after admission, suspected neutropenic sepsis must be treated as an acute medical emergency and empiric antibiotic therapy must be offered. Unfortunately there have been no studies done in Zambia on neutropenic sepsis in cancer patients indicating the common types of organisms and their sensitivity patterns. Therefore, there is no evidence supporting the current choice of empirical antibiotic treatment for neutropenic patients. This might be contributing to morbidity and mortality as neutropenic sepsis could be inappropriately managed. There is, therefore, need to determine the causative organisms of neutropenic sepsis in cancer patients at UTH and their sensitivity patterns to antibiotics. This could thus enable appropriate and optimal management of bacteraemia in neutropenic cancer patients in UTH.

With the dynamic and changing sensitivity pattern towards gram positive organisms, this study will, therefore, define the currently prevailing bacteria causing neutropenic sepsis and their antimicrobial susceptibility pattern in paediatric cancer patients. This will thus contribute to the management of these children and subsequently reduce severe morbidity and mortality associated with neutropenia resulting from wrong antibiotic
choice. In addition, the study will help formulate an evidence-based protocol for the oncology unit.

1.3 Study Question

What are the prevalent bacterial isolates in neutropenic paediatric cancer patients with sepsis at the University Teaching Hospital in Lusaka?

1.4 Objectives

1.4.1 Main objective

To determine the prevalence and pattern of bacterial isolates in paediatric cancer patients with neutropenic sepsis admitted to Oncology ward at the University Teaching Hospital in Lusaka.

1.4.2 Specific Objectives

1.4.2.1 To define the type of organisms isolated in neutropenic paediatric cancer patients.

1.4.2.2 To determine the drug sensitivity patterns of bacterial isolates.

1.4.2.3 To establish the appropriate empiric antimicrobials in treating paediatric cancer patients with suspected neutropenic sepsis.
CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Background

Potent anticancer therapy has increased survival rates of paediatric cancer patients. However, the risk of infection has become even higher as the treatment induces profound and protracted neutropenia (Kim et al., 2005). The World Health Organisation (WHO) defines neutropenia as an absolute neutrophil count of less than 2000/mm³.

Neutropenia is a granulocyte disorder characterised by an abnormally low number of neutrophils, which is the most important type of WBC and serves as the primary defence against infections. The British National Cancer Institute (NCI-CTCAE) delineates neutropenia into four grades according to the absolute neutrophil count (ANC):

- Mild neutropenia (grade 1); ANC of 1,500/mm³ to less than 2000/mm³
- Moderate neutropenia (grade 2); ANC of 1000/mm³ to less than 1,500/mm³
- Severe neutropenia (grade 3); ANC of 500/mm³ to less than 1000/mm³
- Life threatening (grade 4); ANC of less than 500/mm³

Neutropenia results in an increased susceptibility to bacterial infections. The degree of risk depends upon the cause and the severity of the neutropenia, the underlying medical condition or absence of bone marrow reserves for the production of neutrophils. Infection is likely when the neutrophil count falls below 1000/mm³ with escalating risk
at less than 500/mm$^3$ and at less than 100/mm$^3$. Neutropenia can result in bacteraemia which is a condition characterized by the presence of viable bacteria in the blood. The immune system’s response to the presence of bacteria in the blood can cause sepsis, which is an inflammatory immune response of the body.

### 2.2 Clinical Manifestations

Diagnosis of Neutropenic sepsis is made in patients receiving anticancer treatment who develop neutropenia and other signs and symptoms consistent with sepsis. However, signs of neutropenic sepsis are often blurred and often there is no focus of infection. The National Institute for Health and Care Excellence (NICE) guidelines for 2010 states that neutropenic sepsis should be suspected in patients having anticancer treatment who become unwell. Neutropenic sepsis is suspected when a patient becomes ill seven to ten days post chemotherapy which is the classic time for neutropenia. However, delayed neutropenia of up to twenty-eight days can occur with some chemotherapy regimens. Early signs of neutropenic sepsis include a child who generally feels unwell, body temperature of 38 degrees centigrade at any time or 37.4 degrees centigrade on two separate readings an hour apart, shivering, and rigors. Late signs of neutropenic sepsis are hyperthermia or hypothermia, cold and clammy limbs, tachycardia, tachypnoea, restlessness, anxiety or confusion. According to Asturias (2010), the appearance of clinical signs of sepsis in any kind of infection is closely associated with the absolute neutrophil count and a level below 100cell/mm$^3$ is closely associated with a positive blood culture.
2.3 Causative Organisms of Neutropenic Sepsis

Bacterial infections in cancer patients develop quickly, especially in the neutropenic patient, and account for 85-90% of the micro-organisms associated with neutropenia that is accompanied by fever. The most serious neutropenic septic episodes occurred in infections attributed to gram negative organisms such as Enterobacteriaceae or Pseudomonas aeruginosa, and these gram negative bacteria caused approximately 70% of blood stream infections while there were few gram positive bacteria causing bacteraemia in patients with neutropenia. However, infections from gram positive organisms such as Staphylococcus, Streptococcus, Corynebacteria, and Clostridia had increased in the 1990s. This was probably due to the increased use of long indwelling intravascular devices, fluoroquinolone prophylaxis, and high dose chemotherapy induced mucositis. Listeriosis, a severe bacterial infection caused by Listeria monocytogenes, is another infection on the increase in cancer patients (Younger, 2011).

Over the past decades, considerable changes have occurred in the type of bacteria causing infection in cancer patients with neutropenia. According to findings by Zinner (1999), approximately 70% of bacteria isolates from most cancer centres were gram-positive cocci. Similar findings by Feld (2008) noted an increase of gram positive pathogens in most developed countries due to the use of prophylaxis with cotrimoxazole and fluoroquinolones. However, gram negative pathogens are still being isolated in developing countries such as Malaysia and Lebanon and remain high. Additional organisms are anaerobes that account for 0.5-9% of all bacteraemia in hospitalised patients, with variations according to geographical location, demographic
characteristics, most notably age, but few data are available for cancer patients (Zahar et al., 2005).

Trends on causative organisms of infections were studied in a series of 288 episodes of bacteraemia in neutropenic cancer patients in Spain. Incidence of bacteraemia was noted to be increasing significantly from 20 episodes per 1000 admissions in 1986 to 50 episodes per 1000 admissions in 1993 (p=0.00001). There was a continuous increment in gram positive bacteria in neutropenic sepsis (Gonzalez-Barca et al., 1996).

In a similar study done in Belgium by Klastersky et al. (2007), 23% of all patients with febrile neutropenia had bacteraemia. Of these 23% febrile neutropenic episodes, the relative frequency of gram-positive, gram-negative and polymicrobial bacteraemias was 57%, 18% and 13%, respectively.

Similar findings were noted in a study done by van de Wetering (2001) in South Africa. He found that of the 200 episodes of bacteraemia in neutropenic cancer patients, 70% were caused by gram-positive organisms and 20% by gram-negative organisms. Organisms associated with high mortality were gram-negative organisms such as Acinetobacter species, Pseudomonas aeruginosa, and Klebsiella species. However, a study done in Malawi on the causative organisms of bacteraemia, the isolated organisms were gram negative organisms that included Pseudomonas aeruginosa, Escherichia coli, and Salmonella, with sensitivities to ciprofloxacin and cefotaxime. (Israels et al., 2009).

Of concern is that antimicrobial-resistant gram-positive organisms are becoming increasingly frequent in patients with neutropenia. In addition, fluoroquinolone resistant Escherichia coli are being isolated from several cancer centres. A clinical study of
Bacteraemia in Haematological Malignancies was conducted by Fu-Der Wang et al (2005) in Taiwan from 1999-2003 on the aforementioned. The findings were that gram-negative bacilli were still predominant and accounted for 78.2% of isolates, followed by gram-positive cocci (20.8% of isolates), and anaerobes were the least (1% of isolates). *Escherichia coli* was the most common isolated organism accounting for 27.5% of gram-negative bacilli isolates. Other isolates included *Klebsiella pneumoniae* (19.3%), *Pseudomonas aeruginosa* (11%), and *Enterobacter cloacae* (10.1%). Yet other studies cited above particularly in Europe, have demonstrated a predominance of gram positive organisms.

Blood culture isolates from general patients at the University Teaching Hospital microbiology laboratory in Lusaka for the year 2011 showed that the common isolates were *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Escherichia coli*. *Klebsiella pneumoniae* and *Escherichia coli* were isolated from all the departments, while Salmonella was usually from paediatric patients in general. Of concern was the general resistance pattern to commonly used antibiotics with resistance to penicillin of 91.6%, cefotaxime 77.8%, and ciprofloxacin 61.9% (Chileshe-Musyani, 2011).

Studies have shown that there is variation in type of micro-organisms isolated at institutions. Therefore, an appropriate evaluation of empiric antibiotic regimen must be individualised at each institution to maximize benefits for neutropenic cancer patients.
2.4 Management of Neutropenic Sepsis

Empiric antibiotic therapy for neutropenic sepsis must be individualised at each institution due to variations in the causative organisms as noted above. Local bacterial isolates and resistance patterns are crucially important in determining first choice of empiric intravenous therapy for neutropenic sepsis.

Standard management of neutropenic sepsis involves prompt administration of empiric broad spectrum intravenous antibacterial therapy until the neutrophil count has recovered and fever has abated, with additional supportive care (intravenous fluid, oxygen, etc.) as indicated. Guidelines issued by The Infectious Diseases Society of America in 2002 recommended the use of particular combination of antibiotics in specific settings where low risk cases may be treated with a combination of oral cotrimoxazole and ciprofloxacin, while more severe cases require intravenous cefalosporins. If neutrophil count is $\geq 0.5 \times 10^9/L$, the patient is asymptomatic and has been afebrile for 48 hours and blood cultures are negative, antibiotics can be discontinued. If the neutrophil count is $<0.5 \times 10^9/L$, the patient has suffered no complications and has been afebrile for 5-7 days, antibiotics can be discontinued except in certain high risk cases such as acute leukaemia and neutropenic sepsis following high dose chemotherapy when antibacterials are often continued for up to 10 days or until neutrophil count is $\geq 0.5 \times 10^9/L$. (Marti et al, 2009).

Inappropriately treated neutropenic sepsis can have adverse outcomes. A study was conducted by Spanik et al (1998) at St. Elizabeth Cancer Institute, Slovak Republic to assess the outcome of inappropriately treated cancer patients with documented
bacteraemia. Overall, mortality was significantly lower in the patients who were treated appropriately. The reasons noted for inappropriate therapy were selection of the wrong antimicrobials, too short duration of therapy, delayed onset of therapy, or absence of antimicrobial therapy. The conclusion was that inappropriately treated cancer patients with bacteraemia had outcomes that were significantly worse than patients who were treated appropriately.

Israeli researchers observed that improved survival in this population was demonstrated only when antibiotic and antifungal prophylaxis was used in conjunction with barrier isolation. The relative risk for mortality was 0.66 in patients who were given prophylaxis versus relative risk of 0.93 when antimicrobial prophylaxis was not used. In addition, it was noted that the beneficial effect of antimicrobials have not decreased in recent years versus older studies. The beneficial effect of antimicrobials was further emphasised by the Unit of Infectious Diseases at Rabin Medical Centre in Petah-Tikvah, Israel, based on their findings of an overall reduction in case mortality rates in neutropenic cancer patients in 2003.

A high prevalence of resistance to first line drugs has been documented among common bacterial pathogens in the Alliance for the Prudent Use of Antibiotics report entitled “Shadow Epidemic”, 2005. The prevalence of penicillin resistant streptococci is increasing worldwide, ranging from 20% in the United States to more than 50% in Asia and 38% in South Africa (Kathleen et al., 2005).

A study was done in Kenya by Berkley et al (2005) on the prevalence and outcome of bacteraemia among 19,339 children. The investigators found out that the major causes of
death were common bacterial infections, not the often touted culprits-malaria, AIDS, and tuberculosis. The study emphasised the need to pay more attention to common bacterial causes of life-threatening infections in the developing world and knowing the organisms’ susceptibility to antibiotics to help in choosing the appropriate treatment for these patients. However, the article left unanswered the question to which antibiotics the isolates were susceptible or resistant to.

There is a considerable lack of data on antimicrobial susceptibilities in neutropenic cancer patients on chemotherapy in sub-Saharan Africa, including Zambia. Choice of antibiotic treatment must be based on local guidelines which are guided by prevalence of micro-organisms, known resistance patterns and, especially in resource limited countries, affordability (Israels et al., 2009). At the University Teaching hospital, cancer patients with suspected neutropenic sepsis are empirically started on cefalosporins, septrin prophylaxis, and fluconazole. There is therefore need to conduct studies to determine the antimicrobial susceptibilities in neutropenic cancer patients on chemotherapy here in Zambia at the national paediatric cancer referral unit in the University Teaching Hospital in Lusaka.
CHAPTER 3

3.0 STUDY METHODOLOGY

3.1 Study Design

This was a descriptive and analytical cross-sectional study approach involving cancer patients admitted to the paediatric wing of the University Teaching Hospital in Lusaka, Zambia.

3.2 Study Site

The study was carried out at the Oncology ward in the Department of Paediatrics and Child Health at the University Teaching Hospital in Lusaka, Zambia.

The University Teaching Hospital in Lusaka is the largest hospital in Zambia. It serves as the country’s specialist centre receiving cancer referrals from all over the country. The UTH paediatric oncology unit is the only institution offering treatment for cancer, and provides both inpatient and outpatient care. The inpatient facility has a 32 bed capacity. All children admitted to the paediatric admission ward are offered provider initiated testing and counselling for HIV by trained counsellors as part of patient routine care.
3.3 Study Population

The study population comprised of paediatric cancer patients aged 15 years and below admitted to the oncology ward in the Department of Paediatrics and child Health at the University Teaching Hospital in Lusaka. These were patients who developed neutropenia following chemotherapy and presented with symptoms and signs suggestive of neutropenic sepsis.

3.4 Sample Size

The sample size was calculated using the following formula for estimating a proportion with a specified precision (Peacock et al., 2011):

\[ n = \frac{Z^2 \times P(1-P)}{d^2} \]

Where:

- \( n \) = sample size
- \( P \) = expected population proportion.
- \( d \) = desired width of the confidence interval
- \( Z \) = \( Z \) score at 90% confidence interval

Taking expected prevalence of bacteraemia in neutropenic cancer patients to be 25%, (Rapoport, 2011).
A desired width of the confidence level of 0.10, a z score at the 90% confidence interval of 1.645 and assuming 15% non-response rate gives a sample size of 233. That is

\[ N = \frac{1.645^2 \times 4(0.25)(1-0.25)}{(0.10)^2} \]

\[ N = 202 \]

15% non-response = 0.15 x 202 = 30.2 non-response.

Therefore, total sample size is 202 + 30.2 = 232.2 (about 233 participants).

### 3.5 Study Duration

The study was carried out from December, 2014 to May, 2015.

### 3.6 Selection Criteria

Neutropenic paediatric cancer patients on chemotherapy with suspected bacteraemia were enrolled. A convenient sampling method was used. The selection of participants was as follows:

#### 3.6.1 Inclusion Criteria:

- Paediatric cancer patients on chemotherapy with suspected neutropenic sepsis.
- Age 15 years and below
- Consent to enrol into the study by parent or guardian. Assent by paediatric patients aged eight years and above.
3.6.2 Exclusion Criteria

- Children whose parents or guardians declined to participate in the study and lack of assent where applicable.
- Age more than 15 years.

3.7 Data collection

The investigator explained the purpose of the study and obtained consent from parents or guardians, and assent from children aged eight years and above.

A researcher administered standardised questionnaire was used to collect information for each patient. These included demographic characteristics such as age, sex and clinical data. Other relevant information about the diagnosis and antibiotics that had been prescribed for the patient was obtained from the attending physician and from the patient’s file. A data capture sheet was used to record the results of the laboratory findings on the FBC, the absolute neutrophil count and blood culture.

3.8 Sample Collection and Handling

Samples were collected by the investigator as soon as neutropenic sepsis was suspected as part of routine clinical care of the patient. Aseptic techniques were used for blood collection by applying povidone iodine and 70% alcohol at the site of venepuncture. Two millilitres (2 mls) of venous blood was drawn from the antecubital fossa or femoral vein. One ml was inoculated into a BD Bactec-Paediatric Plus blood culture bottle for culture and sensitivity and the remaining 1ml was put in an EDTA specimen bottle for
full blood count and differential white cell count. The specimens were transported immediately to the paediatric laboratory for full blood count, and bacteriology laboratory for blood culture and sensitivity analysis. In case of delay, the samples were stored at room temperature for blood culture and refrigerated at temperature between 2-8°C in case of the full blood count sample.

Identification of positive cultures was done using routine conventional methods by morphological and biochemical tests according to standard operation procedures in use at the University Teaching Hospital microbiology laboratory. The systems used were the API20E system and analytical profile index, which is a miniaturised panel of biochemical tests compiled for identification of groups of closely related bacteria.

Susceptibility testing methods using commercially manufactured disks of antimicrobial agents were used on positive blood culture samples and interpreted according to the University Teaching Hospital microbiology laboratory standards.

The HIV test was offered to all the patients at admission and was carried out by the counsellors as part of routine patient care at the hospital.

### 3.9 Data Management and Analysis

Patients under study were assigned study numbers. The information obtained was entered using Epi info version 7 for windows.

#### 3.9.1 Measurement of Variables

The following were the measured variables of the patients that were enrolled:
3.9.1.1 Dependent/outcome Variable

- Bacteraemia
- Antibiotic sensitivity

3.9.1.2 Independent Variables

- Age
- sex
- Type of cancer
- Clinical symptoms and signs of sepsis
- Neutropenic fever
- Level of neutropenia: mild, moderate, severe, or life-threatening
- HIV status

3.9.2 Data Analysis

Statistical analysis was performed using standard tests. Fisher’s exact test was applied when two or more set of variables were compared. The Mann–Whitney U test was applied instead of the Independent Samples T-test in cases where the sample size in one group of interest was small (less than 30). A p-value of less than 0.05 was considered statistically significant.

The relationship between study variables and bacteraemia was examined using logistic regression. The selection for entry into the logistic regression model was considered at
level p<0.20 or known clinical significance. The p-value, odds ratio, and 95% confidence interval were reported. Statistical analyses were performed using IBM SPSS Statistics version 21.0.

3.10 Ethical Considerations

The study was approved by the ERES Converge Institutional Review Board of Lusaka. The participants were enrolled on a voluntary basis following detailed informed consent explaining the purpose of the study and the pain of venepuncture. Strict confidentiality was maintained at all times by the use of identity study numbers during the study period and where names were used for ease of identification of results in the laboratory, strict confidentiality was ensured and the participants rights were respected at all times. HIV counselling and testing was done by the counsellors according to the hospital’s standard of care. The results of the investigation were made available to the patients and treating physician as soon as they were ready and kept in the patients’ hospital files. The blood culture and sensitivity patterns implied better management of patients with neutropenic sepsis.
CHAPTER 4

4.0 RESULTS

There were 100 neutropenic episodes in paediatric cancer patients with suspected bacteraemia for whom blood cultures were taken. Five of 100 (5%) blood cultures were positive. The following are the characteristics of the neutropenic paediatric cancer patients with suspected bacteraemia.

4.1 Age

The mean age was 7.7 years (SD ± 4.11) and the median age was 6 years. The age range was 1-15 years.

Figure 1 shows the age distribution of the neutropenic cancer patients with suspected bacteraemia.

Figure 1: Age distribution of the patients
4.2 Sex

Figure 2 shows the sex distribution. There were numerically more male children than female children studied, 54/100 (54%) versus 46/100 (46%). However, this difference in proportion was not statistically significant with P-value of 0.42.

Figure 2: Sex distribution of the patients

Source: study findings
4.3 Type of cancer

The two most frequent types of cancers observed in the study children were Leukaemia 30/100 (30%) and Lymphoma 27/100 (27%), (figure 3).

Figure 3: Types of cancer in the patients

Source: Study findings
Table 1 shows clinical stages of cancer and of the study, 62/100 (62%) had known clinical stage of cancer. Of these, 52/62 (84%) had clinical stage IV, 8/62 (13%) had stage III, and 2/62 (3%) had stage I.

Table 1: Clinical stage of the cancer

The table below illustrates the distribution of the clinical stage of cancer.

<table>
<thead>
<tr>
<th>Clinical stage of cancer</th>
<th>Frequency</th>
<th>Per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Stage III</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Stage IV</td>
<td>52</td>
<td>52</td>
</tr>
<tr>
<td>Unknown</td>
<td>38</td>
<td>38</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Source: study findings

4.4 HIV status

The majority of the study population was HIV negative (table 2). Of the 100 neutropenic episodes, 5/100 (5%) were HIV positive. Table 2 below shows the data in this regard.

Table 2: HIV status of the patients

<table>
<thead>
<tr>
<th>HIV status</th>
<th>Frequency</th>
<th>Per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Negative</td>
<td>95</td>
<td>95</td>
</tr>
<tr>
<td>Unknown</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Source: study findings
4.5 Signs and symptoms of infection

The most common sign of infection was fever with 56/100 (56%), followed by tachycardia (table 3).

Table 3: Signs and symptoms of infection

<table>
<thead>
<tr>
<th>Sign/symptom of infection</th>
<th>Frequency</th>
<th>Per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>56</td>
<td>56</td>
</tr>
<tr>
<td>Tachypnoea</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Tachycardia</td>
<td>39</td>
<td>39</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Vomiting</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Shock</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

Source: study findings

4.6 Antimicrobials prescribed for the patient

Seventy three per cent (73%) had a history of having been prescribed antibiotics within seven days prior to presenting with suspected bacteraemia. The antibiotic frequently prescribed was a cephalosporin in 60/73 (82.2%), and cefotaxime topped the list. Other prescribed cephalosporins were ceftriaxone and cephalexin.

Table 4: Antimicrobials prescribed for the patient

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Frequency</th>
<th>Per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzyl penicillin</td>
<td>6</td>
<td>8.2</td>
</tr>
<tr>
<td>Cephalosporin</td>
<td>60</td>
<td>82.2</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>4</td>
<td>5.5</td>
</tr>
<tr>
<td>Other</td>
<td>3</td>
<td>4.1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>73</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

Source: study findings
4.7 Level of neutropenia

The bar chart, figure 4, illustrates the level of neutropenia. Of the neutropenic episodes studied, 78/100 (78%), had level of neutropenia < 1,000, with moderate neutropenia having the least number, 8/100 (8%).

Figure 4: Level of neutropenia in the patients

Source: study findings
4.8 Time interval from last chemotherapy to the time to developing neutropenia

Forty-two out of 100 neutropenic episodes (42%) presented with neutropenia seven to 14 days from the last chemotherapy received. Eight (8/100) had neutropenia three weeks from the last chemotherapy.

Table 5: Time to developing neutropenia

<table>
<thead>
<tr>
<th>Days from last chemotherapy</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;7</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>7 to 14</td>
<td>42</td>
<td>42</td>
</tr>
<tr>
<td>15 to 21</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>22 to 28</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Source: study findings
4.9 Bacterial isolates

There were five (5) positive blood cultures. The gram negative bacterial isolates were four out of the five positive blood cultures (80%), while gram positive bacterium isolate accounted for 20 per cent.

Table 6: Bacteria isolates from blood cultures

<table>
<thead>
<tr>
<th>Bacteria isolated</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>Enterobacter agglomerans</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>Total bacterial isolate</td>
<td>5</td>
<td>100</td>
</tr>
</tbody>
</table>

Source: study findings

The bacterial isolates were subjected to drug sensitivity testing using laboratory standards used in the microbiology laboratory at the University Teaching Hospital.
4.10 Resistance pattern of bacteria isolates

The resistance patterns of the five bacterial isolates are shown in the table below.

Table 7: Resistance patterns of bacteria isolates

<table>
<thead>
<tr>
<th>ISOLATED BACTERIA</th>
<th>AMP</th>
<th>CZM</th>
<th>CHL</th>
<th>CLN</th>
<th>CTX</th>
<th>GEN</th>
<th>IMP</th>
<th>OXA</th>
<th>PEN</th>
<th>SXT</th>
<th>TET</th>
<th>CFT</th>
<th>ACV</th>
<th>CIP</th>
<th>SBM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterobacter agglomerans (n=1)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Klebsiella pneumoniae (n=1)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa (n=1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Escherichia coli (n=1)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Staphylococcus aureus (n=1)</td>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Total number of resistant Isolates</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Resistance Percentage (%)</td>
<td>60</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>20</td>
<td>20</td>
<td>40</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td>60</td>
</tr>
</tbody>
</table>

**Source:** study findings

Abbreviations: AMP, ampicillin; CZM, ceftazidime; CHL, chloramphenicol; CLN, clindamycin; CTX, cefotaxime; GEN, gentamicin; IMP, imipenem; OXA, oxacillin; PEN, penicillin; SXT, cotrimoxazole; TET, tetracycline; CFT, ceftriaxone; ACV, amoxiclav; CIP, ciprofloxacin; SBM, sulbactam.
Of the four gram negative bacterial isolates, two (50%) were resistant to cefotaxime, gentamicin, and ciprofloxacin. In the case of gram positive isolate, *Staphylococcus aureus*, there was resistance to penicillin, oxacillin and ciprofloxacin.

### 4.11 Sensitivity Pattern of Bacterial Isolates

The sensitivity patterns of the five bacterial isolates are shown in the table below.

Table 8: Sensitivity pattern of bacterial isolates

<table>
<thead>
<tr>
<th>ISOLATED BACTERIA</th>
<th>AMP</th>
<th>CZM</th>
<th>CHL</th>
<th>CLN</th>
<th>CTX</th>
<th>GEN</th>
<th>IMP</th>
<th>OXA</th>
<th>PEN</th>
<th>SXT</th>
<th>TET</th>
<th>CFT</th>
<th>ACV</th>
<th>CIP</th>
<th>SBM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterobacter agglomerans (n=1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Klebsiella pneumoniae (n=1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudomonas aeruginosa (n=1)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Escherichia coli (n=1)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus (n=1)</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total number of sensitive isolates</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Sensitivity Percentage (%)        | 20  | 40  | 20  | 40  | 40  |     |     |     |     | 20  | 20  | 40  | 20  |     |     |

**Source:** study findings

Abbreviations: AMP, ampicillin; CZM, cefazidime; CHL, chloramphenicol; CLN, clindamycin; CTX, cefotaxime; GEN, gentamicin; IMP, imipenem; OXA, oxacillin; PEN, penicillin; SXT, cotrimoxazole; TET, tetracycline; CFT, ceftriaxone; ACV, amoxiclav; CIP, ciprofloxacin; SBM, sulbactam.
Of the four gram negative bacterial isolates, two (50%) were sensitive to ciprofloxacin and gentamicin. None of the gram negative isolates were sensitive to cefotaxime. *Staphylococcus aureus* (a gram positive bacterium) was sensitive to chloramphenicol and clindamycin.

### 4.12 Bivariate analysis

At 5% significance level, the patient’s clinical status was significant (P-value < 0.01). All the other patient variables were not statistically significantly associated with bacteraemia at 5% significance level. Table 10 shows the bivariate analysis results for study variables association with bacteraemia.

Table 9: Characteristics of patients with and without bacteraemia

<table>
<thead>
<tr>
<th>Variable</th>
<th>No Bacteraemia</th>
<th>Bacteraemia</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>50</td>
<td>52.6%</td>
<td>4</td>
</tr>
<tr>
<td>Female</td>
<td>45</td>
<td>47.4%</td>
<td>1</td>
</tr>
<tr>
<td>Type of Cancer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphoma</td>
<td>26</td>
<td>27.4%</td>
<td>1</td>
</tr>
<tr>
<td>Rhabdomyosarcoma</td>
<td>13</td>
<td>13.7%</td>
<td>0</td>
</tr>
<tr>
<td>Retinoblastoma</td>
<td>11</td>
<td>11.6%</td>
<td>0</td>
</tr>
<tr>
<td>Neuroblastoma</td>
<td>7</td>
<td>7.4%</td>
<td>1</td>
</tr>
<tr>
<td>Nephroblastoma</td>
<td>8</td>
<td>8.4%</td>
<td>0</td>
</tr>
<tr>
<td>Kaposi Sarcoma</td>
<td>2</td>
<td>2.1%</td>
<td>0</td>
</tr>
<tr>
<td>Brain Tumor</td>
<td>1</td>
<td>1.1%</td>
<td>0</td>
</tr>
<tr>
<td>Leukaemia</td>
<td>27</td>
<td>28.4%</td>
<td>3</td>
</tr>
<tr>
<td>Clinical stage of cancer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage I</td>
<td>2</td>
<td>3.3%</td>
<td>0</td>
</tr>
<tr>
<td>Stage III</td>
<td>8</td>
<td>13.1%</td>
<td>0</td>
</tr>
<tr>
<td>Stage IV</td>
<td>51</td>
<td>83.6%</td>
<td>1</td>
</tr>
<tr>
<td>HIV status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>5</td>
<td>5.4%</td>
<td>0</td>
</tr>
<tr>
<td>Negative</td>
<td>88</td>
<td>94.6%</td>
<td>5</td>
</tr>
<tr>
<td>Critical condition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>13</td>
<td>14.1%</td>
<td>4</td>
</tr>
</tbody>
</table>
### Variable

<table>
<thead>
<tr>
<th>Variable</th>
<th>No Bacteraemia</th>
<th>Bacteraemia</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>79</td>
<td>1</td>
<td>20.0%</td>
</tr>
<tr>
<td><strong>Level of neutropenia</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 500</td>
<td>36</td>
<td>3</td>
<td>60.0%</td>
</tr>
<tr>
<td>500 - &lt; 1,000</td>
<td>38</td>
<td>1</td>
<td>20.0%</td>
</tr>
<tr>
<td>1,000 - &lt; 1,500</td>
<td>8</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>1,500 - &lt; 2,000</td>
<td>13</td>
<td>1</td>
<td>20.0%</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n, mean rank, sum of ranks</td>
<td>95, 49.9, 4742.5</td>
<td>5, 61.5, 307.5</td>
<td>0.38 $^m$</td>
</tr>
</tbody>
</table>

$^*$= Fisher’s exact test, $^m$=Mann-Whitney test

Source: study findings

Analysis of the study population showed that there was generally no statistical difference between the variables except for the clinical condition of the patient (p<0.01). Of the five patients with proven bacteraemia, four were in a critical condition. Males accounted 80% of the patients with proven bacteraemia (p=0.12).

### 4.13 Logistic regression analysis

Since only the patient’s clinical status had a p-value of <0.20 among the study variables, logistic regression was applied to further analyse clinical status of patient in relation to bacteraemia. Compared to non-critical condition patients, critical condition patients had on average 24 times increased odds ratio for bacteraemia (odds ratio=24.31, 95% confidence interval=2.52-234.92, P-value=0.006). Table 13 shows the logistic regression analysis results predicting bacteraemia.
Table 10: Logistic regression predicting bacteraemia

<table>
<thead>
<tr>
<th>Step 1&lt;sup&gt;a&lt;/sup&gt;</th>
<th>B</th>
<th>S.E.</th>
<th>Wald</th>
<th>df</th>
<th>Sig.</th>
<th>Exp(B)</th>
<th>95% C.I.for EXP(B)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower</td>
</tr>
<tr>
<td>Condition of Patient(1)</td>
<td>3.191</td>
<td>1.157</td>
<td>7.600</td>
<td>1</td>
<td>.006</td>
<td>24.308</td>
<td>2.515</td>
</tr>
<tr>
<td>Constant</td>
<td>-4.369</td>
<td>1.006</td>
<td>18.853</td>
<td>1</td>
<td>.000</td>
<td>.013</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Indicates step in the logistic regression model.
CHAPTER 5

5.0 DISCUSSION

Infection is one of the important causes of morbidity and mortality encountered during the treatment of paediatric cancer patients (Paganini et al., 1998). As newly developed more potent chemotherapeutic agents are widely used and broad-spectrum antibiotics are increased, the trends of infections in neutropenic paediatric cancer patients are changing, (van de Wetering et al., 2001). During this study period, a total of 100 neutropenic episodes with suspected bacteraemia had blood culture samples taken. This figure falls short of the targeted 233 sample size. The reason is that the number of neutropenic paediatric cancer patients with suspected bacteraemia had markedly reduced during the same period. The majority of neutropenic cancer patients were clinically stable despite being neutropenic. The reduction in the number of paediatric cancer patients with suspected bacteraemia was attributed to the intensification of infection preventive measures on the oncology ward and the use of cefotaxime, cotrimoxazole and fluconazole prophylactically in neutropenic paediatric cancer patients. Of the patients seen, 73/100 (73%) had been on antibiotics. This affected the number of patients recruited for the study as well as the poor bacterial yield of blood culture samples.
5.1 Characteristics of Patients

The patients enrolled into the study were below the age of 15, with median age of 6 years. This suggests that neutropenic sepsis affects a young population of patients. The number of males (54%) compared to females (46%) showed no statistical difference in proportions with p value of 0.42. The most frequent cancers observed in the study were leukaemias 30/100 (30%) and lymphomas 27/100 (27%). Known clinical stage of the cancer revealed that 52 of the 62 had stage IV cancer (84%). The majority of the children in the study were HIV negative (95%), with 5/100 (5%) being HIV positive. None of the children with positive blood culture were HIV positive.

At enrolment most of the patients had fever (temp >38°C), a late sign of sepsis, accounting for 56% of the study participants. Others were neutropenic patients who were generally unwell and suspected to have sepsis, but had no focus of infection. One patient had pneumonia, while another had cellulitis of the upper limb. This was in keeping with the 2010 NICE Guidelines which states that the signs and symptoms of neutropenic sepsis are often blurred and often there is no focus of infection. Four out of the five patients with positive blood cultures were in a critical condition (p<0.01) with three of the patients having life-threatening neutropenia (absolute neutrophil count of less than 500). This was similar to the findings of Asturias et al, 2010 where they noted an association of neutropenic sepsis with neutrophil count below 100 cells.

The time at which patients presented with neutropenia varied from less than 7 days to more than 3 weeks from the last chemotherapy received. Most of the patients developed neutropenia between 7 to 14 days from last chemotherapy received making up 42/100
(42%) of the patients. This was comparable to NICE Guidelines, 2010 in which the classic time for neutropenia is 7 to 10 days post-chemotherapy. However, delayed neutropenia of up to 28 days does occur. The patients mostly had neutropenia less than 1000, 78/100 (78%).

5.2 Prevalence of Bacteraemia

The prevalence of bacteraemia in neutropenic paediatric cancer patients in this study was 5%. Four out of the five positive blood cultures were nosocomial and one positive blood culture was community acquired. In a South African study, (Rapoport et al, 2011), the prevalence of neutropenic sepsis was 25% which was higher than was found in this study. However, incidence of bacteraemia varies worldwide ranging from 10 to 27% (OudeNijhuis, 2002). In a study done in Barcelona, Spain by Gudiol et al from 2006 to 2010, overall bacteraemia was 5.6 episodes/1000 hospital stays in patients on quinolone and antifungal prophylaxis. With the widespread use of prophylactic antibiotics and antifungal in neutropenic cancer patients on the oncology ward at the University Teaching Hospital, the prevalence of bacteraemia was very low.

5.3 Bacteraemia and Antibiotic Sensitivity Patterns

In this study, gram negative bacteraemia was higher than gram positive bacteraemia, accounting for four of five isolates (80%) and one of five isolates (20%), respectively. Gram negatives isolated were *Escherichia coli* (1), *Enterobacter agglomerans* (1), *Klebsiella pneumoniae* (1), and *Pseudomonas aeruginosa* (1). The gram positive bacterium isolated was *Staphylococcus aureus*. The gram positive bacteraemia was only a fifth of the total bacteraemia. High incidence of gram negative bacteraemia found was
similar to the study done by Israels et al (2009) in Malawi. Studies have shown that gram negative bacteraemia in developing countries is still high while gram positive bacteraemia occurs more in developed countries due to the use of prophylaxis with cotrimoxazole and fluoroquinolones, and the use of long-indwelling central venous lines (Zinner, 1999). In this study, gram positive bacteraemia was not associated with the use of a central venous catheter.

The resistance of cultured gram negative and gram positive organisms from the paediatric neutropenic cancer patients was prominent to the conventional antibiotics of longstanding use, with resistance to cefotaxime being 40% and that to ciprofloxacin 60%. The bacterial isolate, *Escherichia coli*, was resistant to ciprofloxacin which was comparable to similar studies were fluoroquinolone resistant *Escherichia coli* was being isolated from several cancer centres. The gram negative organisms, *Escherichia coli* and *Pseudomonas* were sensitive to gentamicin. However, gentamicin is not used in neutropenic paediatric cancer patients on the oncology ward at the University Teaching Hospital in Lusaka due to the concomitant use of nephrotoxic antineoplastic agents. None of the gram negative bacterial isolates were sensitive to cefotaxime, and only one (20%) was sensitive to ciprofloxacin. *Staphylococcus aureus* was sensitive to chloramphenicol and clindamycin, drugs that are rarely used in paediatric cancer patients with suspected bacteraemia at the University Teaching Hospital.
CHAPTER 6

6.0 CONCLUSION

Bacteraemia in neutropenic paediatric cancer patients causes morbidity during the treatment of cancer and appears to affect a young population of patients (median age 6 years). Most affected patients were in an advanced stage of their disease. Commonest malignancies involved are leukaemias and lymphomas. Most patients, if not all, are not infected with HIV. The prevalence of bacteraemia in neutropenic sepsis was 5%. The major causative organisms of bacteraemia in neutropenic paediatric cancer patients are gram negatives (80%) which have high antimicrobial resistance to the commonly used antibiotics on the oncology ward at the University Teaching Hospital in Lusaka. The choice of antibiotic in treating neutropenic paediatric cancer patients with suspected bacteraemia needs to take into account the prevalence of gram negative bacteraemia among inpatients and antibiotic resistance of gram negative and gram positive bacteria. The blood culture positivity rate in this study was lower than findings of similar studies in the region which could be attributed to the small sample size and prophylactic use of antibiotics by some participants.

6.1 Limitations of the Study

Limited capacity of the BACTEC machine leading to delays in incubating the samples contributed to the low bacterial yield on blood cultures. The study was done with unanticipated drop in the number of neutropenic paediatric cancer patients with suspected bacteraemia. The sample size of 233 could not be achieved due to the limited
time in which the study was conducted. The final analysis including only 100 episodes of neutropenic sepsis out of the proposed 233 was acceptable for statistical analysis, but significantly reduces the power of the study.

6.2 Benefits of the Study

The study has provided some information on the causative organisms of bacteraemia in neutropenic paediatric cancer patients and the drug sensitivity patterns. The study participants with positive blood cultures had the opportunity of appropriate antibiotic use and thus reducing morbidity and mortality.
7.0 RECOMMENDATIONS

A larger study needs to be carried out with a larger sample size and more rigorous methodology. It is important to select the proper empirical antibiotics prior to blood culture and antibiotic sensitivity test results if bacteraemia is suspected in paediatric cancer patients. There must be judicious use of antibiotics to reduce the emergence of EBLs. Continuous monitoring and periodic analysis of the isolated pathogens with antibiotic sensitivity test results must be carried out at the institution.
REFERENCES


8.0 APPENDICES

8.1 Appendix I

Patient Information Sheet

Title of study: The Prevalence of Bacteraemia in Neutropenic Paediatric Cancer Patients on Chemotherapy at the University Teaching Hospital in Lusaka.

Investigator: Dr. Grace Chingo

Introduction

I am a doctor for children working in the department of paediatrics. I am doing a research for my master’s program. Kindly note that you’re agreeing to participate in this study is voluntary.

Purpose of study

The study is being conducted in view of the fact that cancer patients are susceptible to infections because of the cancer itself and the treatment given for cancer. Cancer treatment usually results in reduction of cells in your blood that help to fight infections resulting in increased susceptibility to infections that can be life-threatening. Various types of bacteria can cause infection and treatment has to be tailored to the causative organism. Knowledge of the common infecting organisms will help health care providers to provide better treatment to cancer patients with infections due to reduction of cells in the blood that fight infections.
Procedure of study

If you agree to participate in this study, we will obtain information from you regarding age of your child and social data. Sample of two millilitres (2ml) of blood will be drawn from your child from the elbow or groin area and taken to the laboratory for testing. One millilitre will be used to check the number of cells in the blood that help fight infections, and another one millilitre to check on the type of bacteria and the antibiotics to which the bacteria are susceptible. Results of the findings in the laboratory will be communicated to you. The doctor looking after your child will be given the results and decide on the best treatment to be given.

Risks

There are no added risks to your child if you participate in this study. However, your child will experience discomfort during blood collection.

Benefits

The information obtained will contribute to help improve the treatment of your child and other children in future who will have similar medical presentation.

Confidentiality

All the information obtained will be strictly confidential. Your name and that of your child will not appear on the study files.

Thank you for considering participating in this study. If you have any queries, concerns or clarifications, please contact Dr. Chingo or The Chairperson ERES Converge IRB on the following addresses:
Dr. G. Chingo

Department of Paediatrics and Child Health

University Teaching Hospital

P. O. Box P/B RW 1X

Lusaka.

Mobile: +260 979 571 039/+260 966 571 039

Email: grchingo93@yahoo.com

The Chairperson

ERES Converge IRB

33 Joseph Mwila Road

Roads Park

Lusaka.

Tel: +260 955 155 633

+260 955 155 634

+260 966 765 503
8.2 Appendix II

Consent Form

Title of study: The Prevalence of Bacteraemia in Neutropenic Paediatric Cancer Patients on Chemotherapy at the University Teaching Hospital in Lusaka.

Investigator: ………………………………………………………………………………………………….

I …………………………………………………have been informed adequately on the reason for this study, the benefits and risks of this study. I am aware that my personal details will not appear in this study and the information obtained will be anonymously processed. Therefore, with full understanding of the importance of this study, I agree to give the requested information and the blood samples in this clinical investigation.

Parent/guardians signature ………………………….. Date …………………

If illiterate (thumb print) ………………………….. Date …………………

Person obtaining information ………………………….. Date …………………

Name of witness ………………………….. Date …………………
8.3 Appendix III

Assent Form

Title of Study: The Prevalence of Bacteraemia in Neutropenic Paediatric Cancer Patients on Chemotherapy at the University Teaching Hospital in Lusaka.

Investigator: ……………………………………………………………………………………………

This is a study looking at the bacteria that cause infections in children following cancer treatment. This will help the health care providers in providing treatment to me and other children with a similar medical problem. I understand that I may feel some discomfort during blood collection. I know that my name will not be included in the report or that I was in this study.

I …………………………………………………………………………………………want to be in this research study.

Signature ………………………………….. Date …………………………………………………
8.4 Appendix IV

Questionnaire

Particulars of Patient

Study ID………………

Hospital no………………

Age …………………………………

Sex (tick)  M …………………  F …………………

Date of birth …………………………………
(DD/MM/YY)

Date of admission …………………………………
(DD/MM/YY)

Date of enrolment …………………………………
(DD/MM/YY)

Residence …………………………………
### Clinical Data

<table>
<thead>
<tr>
<th>Type of cancer</th>
<th>...............................................................</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical stage of cancer</td>
<td>...............................................................</td>
</tr>
<tr>
<td>Chemotherapy regime</td>
<td>...............................................................</td>
</tr>
<tr>
<td>Condition of patient</td>
<td>...............................................................</td>
</tr>
<tr>
<td>(critical/not critical)</td>
<td>...............................................................</td>
</tr>
<tr>
<td>HIV status(tick)</td>
<td>Reactive</td>
</tr>
<tr>
<td>Weight(kg)</td>
<td>.......</td>
</tr>
</tbody>
</table>

### Signs and symptoms of infection

<table>
<thead>
<tr>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperthermia (temperature 38°C or more)</td>
<td>.......</td>
</tr>
<tr>
<td>Tachypnoea</td>
<td>.......</td>
</tr>
<tr>
<td>Tachycardia</td>
<td>.......</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>.......</td>
</tr>
<tr>
<td>Vomiting</td>
<td>.......</td>
</tr>
<tr>
<td>Shock</td>
<td>.......</td>
</tr>
<tr>
<td>Others(specify)</td>
<td>.......</td>
</tr>
</tbody>
</table>
Any focus of infection: Yes ........... No ...........

If yes to above specify…………………………………………………………

**Antimicrobials Prescribed For the Patient**

<table>
<thead>
<tr>
<th>Type of antibiotic</th>
<th>Duration of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzyl penicillin</td>
<td>.................................................................</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>.................................................................</td>
</tr>
<tr>
<td>Cephalosporin</td>
<td>.................................................................</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>.................................................................</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>.................................................................</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>.................................................................</td>
</tr>
<tr>
<td>Others(specify)</td>
<td>.................................................................</td>
</tr>
</tbody>
</table>

**Laboratory Data**

<p>| Date of specimen collection | ................................................................. |
| Total WBC count(x10^3/mm^3) | ................................................................. |
| Differential WBC count      | ................................................................. |</p>
<table>
<thead>
<tr>
<th></th>
<th>Absolute count(x10³/mm³)</th>
<th>In %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eosinophils</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basophils</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelet count(x10³/mm³)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haemoglobin(g/dL)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Culture and Identification of Blood Specimens**

Name of bacteria isolated .................................................................

**Antimicrobial susceptibility testing (tick)**

<table>
<thead>
<tr>
<th></th>
<th>S</th>
<th>R</th>
<th>PS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cefotaxime</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gentamicin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vancomycin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Imipenem</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drug</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Tetracycline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TMP-SXT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Penicillin G</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Other morbidity .................................................................

Name of investigator .................................................................
8.5 Appendix V

15th October, 2014

Ref. No. 2014-Apr-005

The Principal Investigator
Dr. Grace Chingo
University Teaching Hospital
School of Medicine
Dept. of Paediatrics and Child Health
P/B RW1X,
LUSAKA.

Dear Dr. Chingo,

RE: SUSPECTED BACTERAEMIA IN NEUTROPENIC PAEDIATRIC CANCER PATIENTS ON CHEMOTHERAPY AT THE UNIVERSITY TEACHING HOSPITAL IN LUSAKA.

Reference is made to your correspondence dated 10th October, 2014. The IRB resolved to approve this study and your participation as principal investigator for a period of one year.

<table>
<thead>
<tr>
<th>Review Type</th>
<th>Approval Date: 15th October, 2014</th>
<th>Expiry Date: 14th October, 2015</th>
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<tr>
<td>Approval and Expiry Date</td>
<td>15th October, 2014</td>
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<td>Protocol Version and Date</td>
<td>25th April, 2014</td>
<td>14th October, 2015</td>
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<td>Information Sheet, Consent Forms and Dates</td>
<td>English.</td>
<td>14th October, 2015</td>
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<tr>
<td>Consent Form ID and Date</td>
<td>Version: Nil</td>
<td>14th October, 2015</td>
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<tr>
<td>Recruitment Materials</td>
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<td>14th October, 2015</td>
</tr>
<tr>
<td>Other Study Documents</td>
<td>Questionnaire.</td>
<td>14th October, 2015</td>
</tr>
<tr>
<td>Number of participants approved for study</td>
<td>213</td>
<td>14th October, 2015</td>
</tr>
</tbody>
</table>
Specific conditions will apply to this approval. As Principal Investigator it is your responsibility to ensure that the contents of this letter are adhered to. If these are not adhered to, the approval may be suspended. Should the study be suspended, study sponsors and other regulatory authorities will be informed.

Conditions of Approval

- No participant may be involved in any study procedure prior to the study approval or after the expiration date.
- All unanticipated or Serious Adverse Events (SAEs) must be reported to the IRB within 5 days.
- All protocol modifications must be IRB approved prior to implementation unless they are intended to reduce risk (but must still be reported for approval). Modifications will include any change of investigator's or site address.
- All protocol deviations must be reported to the IRB within 5 working days.
- All recruitment materials must be approved by the IRB prior to being used.
- Principal investigators are responsible for initiating Continuing Review proceedings. Documents must be received by the IRB at least 30 days before the expiry date. This is for the purpose of facilitating the review process. Any documents received less than 30 days before expiry will be labelled “late submissions” and will incur a penalty.
- Every 6 (six) months a progress report form supplied by ERES IRB must be filled in and submitted to us.
- ERES Converge IRB does not “stump” approval letters, consent forms or study documents unless requested for in writing. This is because the approval letter clearly indicates the documents approved by the IRB as well as other elements and conditions of approval.

Should you have any questions regarding anything indicated in this letter, please do not hesitate to get in touch with us at the above indicated address.

On behalf of ERES Converge IRB, we would like to wish you all the success as you carry out your study.

Yours faithfully,

ERES CONVERGE IRB

Dr. E. Munalula-Nkandu
BSc (Hons), MSc, MA Bioethics, PgD R/Ethics, PhD
CHAIRPERSON
8.6 Appendix VI

18th May, 2014

Ref. No. 2014-Apr-005

The Principal Investigator
Dr. Grace Chingo
University Teaching Hospital
School of Medicine
Dept. of Paediatrics and Child Health
P/B RW1X,
LUSAKA.

Dear Dr. Chingo,

RE: SUSPECTED BACTERAEAMIA IN NEUTROPENIC PAEDIATRIC CANCER PATIENTS ON CHEMOTHERAPY AT THE UNIVERSITY TEACHING HOSPITAL IN LUSAKA.

We acknowledge receipt of your proposed amendment dated 13th May, 2015.

We take note of your “good problem”- given that there is a reduction of cancer patients with suspected bacteraemia.

This amendment is approved as submitted.

Yours faithfully,
ERES CONVERGE IRB

Dr. E. Munafula-Nkandu
BSc (Hons), MSc, MA Bioethics, PgD R/Ethics, PhD
CHAIRPERSON