THE UNIVERSITY OF ZAMBIA
SCHOOL OF MEDICINE
DEPARTMENT OF PATHOLOGY AND MICROBIOLOGY

VALUE OF PROCALCITONIN AND C-REACTIVE PROTEIN AS EARLY MARKERS OF BACTERAEMIA AMONG PATIENTS WITH HAEMATOLOGICAL MALIGNANCIES RECEIVING CHEMOTHERAPY, LUSAKA, ZAMBIA

A Dissertation Submitted to the University of Zambia in Partial Fulfilment of the Requirement for the Master of Science in Pathology (Haematology)

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DECLARATION

This work/dissertation in its present form has not been submitted or accepted previously for the award of a degree or diploma in this or any tertiary institution, and is not being submitted for a degree or diploma in any tertiary institution or for another degree or diploma at this institution. I declare that this Dissertation contains my own work except where specifically acknowledged. I further declare that I followed all the applicable ethical guidelines in the conduct of the research. This dissertation has been prepared in accordance with the Master of Science in Pathology (Haematology), University of Zambia (UNZA) guidelines.

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CERTIFCATE OF APPROVAL

The University of Zambia approves this Dissertation on *"**Value of Procalcitonin and C-reactive protein as early markers of bacteraemia among patients with haematological malignancies receiving chemotherapy, Lusaka, Zambia**"*.

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ABSTRACT

Introduction: The immune system of patients with haematological malignancies is suppressed during chemotherapy. This renders them vulnerable to frequent infections especially bacterial. Timely diagnosis of these infections is difficult, because a severe infection may be asymptomatic or manifest only in the form of fever or malaise. There is need to come up with laboratory markers that can detect an infectious process at an early stage.

Aim: The aim of this study was to determine the value of using Procalcitonin (PCT) and C reactive protein (CRP), for early diagnosis of infection in patients with haematological malignancies receiving chemotherapy.

Method: This was a cross sectional study consisting of sixty eight (68) patients with haematological malignancies. Data from each participant including sex, age, clinical and laboratory presentation were collected after obtaining informed consent. Then Specimens were collected for measurement of PCT, CRP and for bacteriological analysis. Patients were divided into two groups; those with a bacterial culture positive and negative result. Procalcitonin and CRP concentrations were compared between groups using t-test and non-parametric statistical tests respectively. The area under ROC curve, sensitivity, specificity, likelihood ratio, and Spearman's correlation coefficient also calculated.

Results: A total of 14 (20.6%) microorganisms were isolated, of which 10 were gram-positive bacteria and 4 were gram-negative bacilli. The mean values of PCT which were 6.1ng/mL in the bacteraemia group and 5.1ng/mL in the non-bacteraemia group, p=0.023 and median CRP values were 24.2 (6.43-48.15) in the bacteraemia and 23.5 (6.03-75.44) in the non-bacteraemia group, p=0.832. The area under curves was 0.52 (95% CI=0.57-0.84) for CRP and 0.70 (95% CI=0.35-0.69) for PCT. PCT value of greater than 5.1ng/mL is diagnostic for infections (sensitivity 71%, specificity 65%) while that of CRP was 21mg/mL with the sensitivity and specificity of 64% and 44% respectively. Elevated levels of PCT as well as fever were significantly associated with bacteraemia.

Conclusion: Procalcitonin measurements can be of value in the early diagnosis of bacteraemia in patients with haematological malignancies. In contrast, the diagnostic sensitivity and specificity

for CRP was found to be too low in this study to safely rely on it as a marker of bacteraemia in patients with haematological malignancies.

Key words: Procalcitonin (PCT), C reactive protein (CRP), Haematological Malignancies, Bacteraemia Marker

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LIST OF ABBREVIATIONS

AML – Acute Myeloid Leukaemia

ALL- Acute Lymphoblastic Leukaemia

AUC- Area under ROC Curves

BREC–Biomedical Research Ethics Committee

CRP – C-reactive protein

DRGS – Directorate of Research and Graduate Studies

EDTA– Ethylene diamine tetra acetic acid

ELISA – Enzyme-Linked Immuno-sorbent Assay

FBC- Full Blood Count

ICU – Intensive Care Unit

IL – Interleukin

PCT- Procalcitonin

ROC- Receiver Operating Characteristics

SPSS- Statistical Package for Social Sciences

TNF-α – Tumour Necrosis Factor Alpha

UTH – University Teaching Hospital

UNZA– University of Zambia

VEGF-Vascular Endothelial Growth Factor

WHO – World Health Organizaion

1.0 INTRODUCTION

1.1 Background

Although major advances in the care of patients with malignant cancers over the past several decades have resulted in improved survival, infections remain a significant cause of morbidity and mortality (Zembower *et al.,* 2014). Patients with haematological malignancies are immunocompromised because of both the malignancy and the treatment used to manage it (Hämäläinen, 2010). This renders them susceptible to bacterial and fungal infections (Chandran *et al*., 2012). Early diagnosis is difficult, because a severe infection may be asymptomatic or only manifests as fever or malaise (Chandran *et al*., 2012). Therefore, treatment for these infections is empirical, and broad-spectrum antibiotics and antifungal, are recommended for patients presenting with fever (Alison *et al*., 2011).

Recent advances have resulted in effective chemotherapy drugs and regimens have improved survival rates of these patients. In spite of the obvious improvements, chemotherapy still has certain disadvantages; it induces the breakdown of mucosal barriers, usually leading to high risk of neutropenic fever, which is associated with development of severe infection and high mortality. Thus, early initiation of antimicrobial therapy (Kumar *et al.*, 2006) and other supportive measures are vital in the management of haematological cancer patients receiving intensive chemotherapy.

Fever during chemotherapy-induced neutropenia may be the only indication of a severe underlying infection, because signs and symptoms of infection or inflammation typically are attenuated (Alison *et al*., 2011). Bacterial infections may cause serious complications if left untreated, while treating viral illnesses or non-infective causes of inflammation with antibiotics is not only ineffective, but also contributes to the development of resistance, increases costs, and the risks of toxicity and hypersensitivity reactions (Simon *et al*., 2004).

Haematological malignancies are associated with specific immune defects that predispose to infections with particular pathogens. Patients with acute leukaemia have increased risk of severe gram-negative bacterial infections as a result of quantitative or functional neutropenia. Patients

with chronic leukaemia and multiple myeloma are susceptible to invasive bacterial infections from staphylococci and streptococci, especially pneumococcus. Conversely patients with lymphoma have abnormalities of the cellular immune system resulting in an increased risk of viral infections (e.g. herpes simplex) and fungal infections (e.g. *Cryptococcus*) (Sharma and Lokeshwar, 2005).

Identification of markers for the early recognition of bacterial infections could guide treatments, reduce misuse of antibiotics, and possibly improve long-term outcomes (Velicer *et al*., 2004). Procalcitonin is a 116 amino acid precursor of the hormone calcitonin. It has been shown, that bacterial infections are capable of triggering ubiquitous expression of the calcitonin gene (*CALC-1)*, along with constitutive release of PCT from a number of tissues and differentiated cell types, so that a significant increase of PCT levels can be observed in patients with severe bacterial infection and/or sepsis (Dipalo *et al*., 2014).

C-reactive protein is an acute-phase protein that is synthesized in the liver in response to interleukin 6 and is normally found at concentrations of less than 10 mg/L in the blood. During infectious or inflammatory disease states, CRP levels rise rapidly and its levels are frequently used to aid diagnosis of infections (WHO, 2014). This study will evaluate the value of PCT and CRP, in early diagnosis of infections in patients with haematological malignancies on chemotherapy.

1.2 Statement of the problem

Infectious complications are a serious cause of morbidity and mortality in cancer patients. Autopsy studies show that approximately 60% of deaths are infection related in patients with underlying haematological malignancies (Zembower *et al*., 2014). Patients with haematological malignancies are more likely to be immunocompromised than patients with solid organ malignancy and are at high risk of sepsis and have worse prognosis compared to solid tumours (Williams *et al*., 2004).

One study reported that about 85% of patients administered on induction chemotherapy for acute leukaemia develop life threatening infectious complications. These complications cause death in up to 70% of these patients (Sylvester *et al*., 2003).

The challenge in the management of neutropenic cancer patients with fever arises from the difficulty in distinguishing between infection and the other causes of fever. However, fever remains the most constant and often the only indicator of infection, since patients with neutropenia lack an inflammatory reaction at the site of infection until recovery occurs (Klastersky *et al*., 2004).

Cancer patients with neutropenia have a high risk of developing infectious complications. It has been estimated from other studies that at least 30-60% of neutropenic cancer patients develop an infection, of which 13-37% develop bacteraemia (Perola *et al*., 2005). The likelihood of acquiring nosocomial infections is high especially for cancer patients who are admitted for a long time within the hospital setting.

Early diagnosis of these infections is a challenge because microbiological methods take more than two days to confirm an infection. Furthermore, delayed recognition and/or poor implementation of the appropriate treatment strategy can lead to significant morbidity and mortality (Chandran *et al*., 2012).

1.3 Study Justification

Patients treated with aggressive chemotherapy for malignancy very often develop neutropenia. Fever can develop due to the neutropenia and is usually considered a medical emergency and thus requires prompt evaluation of cause and initiation of empiric therapy. The presence of fever is most often than not presumed to be due to an underlying bacterial infection (Chandran *et al*., 2012). The general consensus in management of these patients is to provide antibiotics for every fever that a patient presents.

The response time of microbiological tests limits the possibility of an early etiologic diagnosis. Early diagnosis and initiation of therapy is more effective than lately initiated therapy with a specific diagnosis (Mesiner, 2010). For this reason it is useful to find sensitive and specific indicators, whose determination is rapid and correlates with the severity and prognosis of the infection, to differentiate between patients with and without infection.

In this context, biological markers would be valuable clinical tools for identifying bacterial infections early. These markers can be useful in settings of little or no laboratory infrastructure since they could also be available as point-of-care (POC) tests. These serum biomarkers have not been reliably proved to diagnose infections (Lehrnbecher and Laws, 2005).

Procalcitonin is routinely reported as both more sensitive and specific than CRP (Casado *et al.*, 2003). Critically elevated PCT levels are nearly patho-gnomonic for bacterial sepsis in non-neutropenic and can be used to guide management (Wacker *et al*., 2013), even though it does not play a major role in several international guidelines (Hentschel *et al*., 2014). Further research is needed to evaluate the performance of these markers in detecting infections in neutropenic cancer patients.

1.4 Literature review

1.4.1 Infectious complications in Haematological malignancies

Patients with haematological malignancies are at increased risk of infections not only because of the malignancy itself but also because of neutropenia induced by intensive chemotherapeutic treatment. Bacteremia is a relatively common serious complication occurring in around 15% of patients with haematological malignancies within the first years after diagnosis but the risk varies between different types of haematological malignancies (Nørgaard, 2012).

A study which analyzed causes of death, other than resistant disease or relapse, in 875 children with acute lymphoblastic leukaemia (ALL) and 229 with acute myeloid leukaemia (AML), reported that, 23 (2.6%) ALL and 44 (19.2%) AML patients died. The main cause of early death was haemorrhage, often associated with hyper-leucocytosis, and infection for chemotherapy related mortality (Slats *et al*., 2005).

Neutropenia is also a common complication of chemotherapy in Hodgkin lymphomas, but related complications including febrile neutropenia (FN), neutropenic sepsis, and death are much less frequent (Vakkalanka and Link, 2011).

Polymorphonuclear leukocytes (PMNs or neutrophils) are a key component of innate innate immunity. Neutrophils are rapidly recruited to sites of bacterial infections by host- and/or pathogen-derived components, which also prime them for enhanced microbicidal activity (Kobayashi *et al*., 2005). They bind and ingest microorganisms by a process known as phagocytosis. Chemotherapy suppresses the hematopoietic system, impairing host protective mechanisms, resulting in neutropenia and allowing bacterial multiplication and invasion (Crawford, 2004).

The incidence and mortality in non-Hodgkin's lymphoma are increasing. Several risk factors for chemotherapy induced neutropenia and its complications (e.g. hospitalization for febrile neutropenia and dose delays or reductions) have been identified in patients with non-Hodgkin's lymphoma treated with standard-dose regimens (Zelenetz *et al.*, 2003).

1.4.3 Use of Procalcitonin in the diagnosis of infections

Much research has focussed on use of PCT as a marker of infection in sepsis. C-reactive protein is widely used as clinical marker of infection but not much is known about procalcitonin in the clinical setting. Data is accumulating supporting the role of PCT as a clinical tool for determining the presence of an underlying bacterial infection in septic patients in non neutropenic patient but fewer data is available for neutropenic cancer patients. A few examples of studies that have been done on these two markers in patients with sepsis have been highlighted here.

Among paediatric ICU patients Cies e*t al* (2014) recently demonstrated that a PCT value of ≥1ng/mL predicted having a serious bacterial infection (OR = 1.18, 95% CI 1.07–1.49) with a sensitivity of 70%, and a specificity of 68% in their study which was done in France. A separate prospective cohort study done in Spain, Rey *et al* (2007) reported that a PCT level of >1.63ng/mL had 85% sensitivity and 83% specificity for determining the presence of sepsis in paediatric ICU patients. They also quantified the positive correlation between disease severity and increasing PCT levels. The median PCT plasma levels of 0.17, 0.43, 0.79, 1.80, 15.40, and 19.13ng/mL in negative, systemic inflammatory response syndrome, localized infection, sepsis, severe sepsis, and septic shock groups, respectively was reported (Rey, 2007)

A study done in China, in which tried to determined whether an initial PCT could assist in assessing the risk of infections in Non-Hodgkin's Lymphoma (NHL), found that an initial PCT value of ≥ 0.50 ng/mL was a high risk for infections with of sensitivity 83.5% and specificity 77.2%) (Xiao *et al*., 2015).

However, a Belgian team found lower sensitivity and specificity for PCT (68% and 23%, respectively) in urinary tract infection diagnosis (Tuerlinckx *et al*., 2005). This probably is because PCT may not perform well in patients with a localized infection like in urinary tract infections. Other studies mentioned above which have evaluated these markers in patients with systemic infections show consistent findings.

1.4.4 Procalcitonin a better marker of infections than C-reactive protein

In recent years measurement of procalcitonin, C-reactive protein and other inflammatory mediators have been reported as sensitive parameters for the early diagnosis of neonatal sepsis and evaluating its outcome (Lachowska *et al*., 2003).

The superiority of PCT against other surrogate markers e.g. CRP has been shown in several studies. Simon *et al*., (2004) performed a meta-analysis of studies that evaluated PCT and CRP for the diagnosis of bacterial infections. He reported that PCT is more sensitive and more specific than CRP in discriminating bacterial from non-infective causes of systemic inflammation. These conclusions were also supported by Hentschel *et al*, (2014).

The role of PCT and CRP in early diagnosis of sepsis in patients with febrile neutropenia was studied among patients with haematological malignancies in study done in Iran. They reported the sensitivity and specificity of 92.5% and 97.3% respectively, for PCT while 70.5% and 42.1% for CRP (Meidani *et al*., 2013). Marshall *et al* (2014) in their study done in Cuba reported that among HIV patients with bloodstream infections, PCT showed higher discriminative ability than CRP to determine the occurrence of bacteraemia. The area under receiver operating curve (AUC) was reported to be 0.47 for CRP and 0.80 for PCT.

In Africa there is limited data available to show the performance of PCT and CRP in patients with an infection. A study by Ali *et al*, (2014) done in Egypt supports the role of PCT in the diagnosis of sepsis among neonates. Their study found that PCT was positive at a cut off 0.5ng/L in 100% of proven septic cases compared to the CRP at cut off of 23 mg/L showed a positivity of 63.6%. Another study in Niger, among patients with Severe Acute Malnutrition with infectious complications, CRP and PCT levels were found to be higher in bacteraemia than in patients with no bacteraemia (p=0 .002) (Page *et al*., 2014). We did not find any study in Southern Africa including Zambia that evaluated these two markers in the diagnosis of infections.

1.5 Research question

Do the levels of PCT and CRP among patients with haematological malignancies on chemotherapy reliably indicate a bacterial infection?

2.0 OBJECTIVES

2.1 General objective

To determine the value of using procalcitonin and C reactive protein, for early diagnosis of bacterial infection in patients with haematological malignancies receiving chemotherapy

2.2 Specific objectives

2.2.1 To determine the levels of PCT and CRP in patients with and those without bacterial infections among patients with haematological malignancies.

2.2.2 To determine the diagnostic sensitivity, specificity and likelihood ratios for both PCT and CRP in predicting bacteraemia among patients with haematological malignancies

2.2.3 To determine the relationship of PCT and CRP in patients with haematological malignancies

2.2.4 To identify bacterial isolates found in patients with haematological malignancies on chemotherapy

3.0 METHODOLOGY

3.1 Study site

The study was conducted at the University Teaching Hospital (UTH) and Cancer Diseases Hospital (CDH) from February, 2016 to July, 2016. Paediatric patients seen from the paediatric oncology ward were included in the study, while adult patients were recruited from the Department of Medicine at the University Teaching Hospital. Other adult patients those with lymphomas were recruited within the various wards of the Cancer Diseases Hospital. Written informed consent was obtained from the patient or the guardian before inclusion in this study.

3.2 Target population

Patients between the ages of 3 to 70 years, diagnosed with haematological malignancies and receiving chemotherapy

3.3. Study population

Patients with a haematological malignancy receiving chemotherapy with neutropenia with or without fever who present to the UTH and CDH

3.4 Study design and algorithm

The study employed a cross sectional design involving 68 patients. CRP, PCT and blood culture were analysed in patients with haematological malignancies on chemotherapy treatment. Patients were evaluated for clinical evidence of infection, and samples collected for processing. Prior to specimen collection the body temperature which was taken by an attending nurse or clinician and recorded on temperature charts, was used for the purpose of this study. Fever was defined as a single body temperature reading higher than 38.0°C according to the 2002 guidelines for the use of antimicrobial agents in neutropenic patients with cancer (Hughes *et al.*, 2002). Neutropenia was defined as leukocytes less than 1.0×10^9/L for adults and 1.5×10^9/L for paediatric patients. Patients with bacteraemia are those with a positive bacterial culture while non-infected patients are those with no bacteriological or clinical signs of infection, and did not receive antibiotics for at least one month. Clinical data were collected from all patients using their patient charts and files. Figure 01 shows the process that was followed from participant recruitment, to specimen collection and procession.

3.4.1 Study algorithm

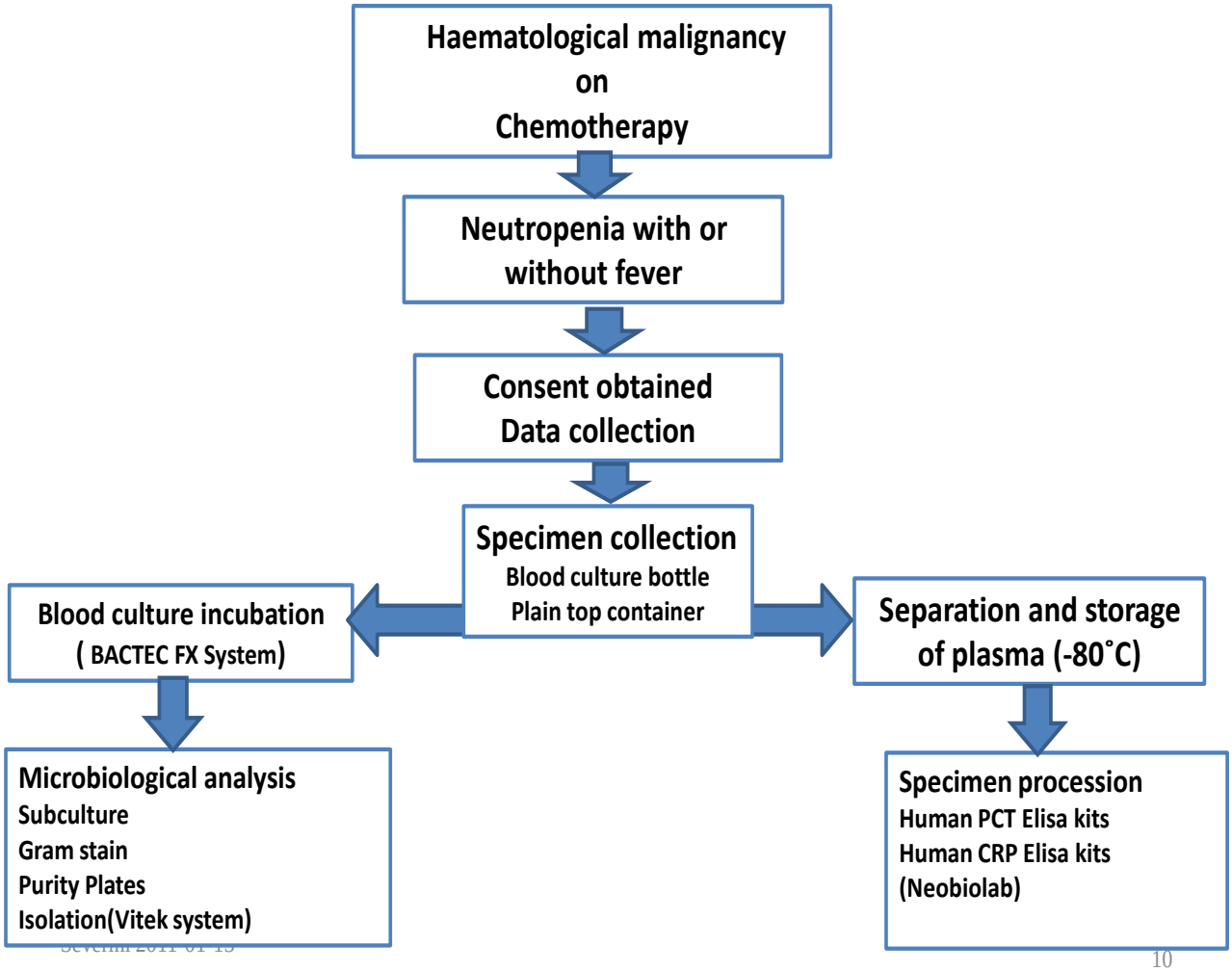

Figure 1: Participant recruitment and specimen collection and procession chart

3.5 Sampling method

Convenience sampling method was employed because these are relatively rare condition; therefore we sampled the available participants as long as they meet the eligibility criteria.

3.6 Sample size

The diagnostic formula was used to calculate sample size; since PCT has a higher sensitivity than CRP (according to literature). Hence, it was used to calculate sample size.

$$TP + FN = Z^2 \times \frac{(SN\ (1-SN))}{W^2} \qquad N(SN) = \frac{(TP + FN)}{P}$$

Where
TP is True positive
FN is False Negative
SN is Sensitivity (from literature at 92%)
Z is confidence interval at 95% confidence i.e. Z=1.96
P is prevalence (5.7% worldwide prevalence of haematological malignancies)
W is accuracy = 0.05

$$TP + FN = (1.96)^2 \times \frac{\{0.92(1-0.92)\}}{(0.05)^2} = 3.842 \times \frac{0.0736}{0.0025} = \underline{113.11}$$

$$N(SN) = \frac{(TP + FN)}{P} = \frac{113.11}{0.057} = 1{,}984$$

Therefore N(SN)= 1,984

The estimated number of patients with haematological malignancies is 69 participants. This was discovered after a review of records at the university teaching hospital and the cancer diseases which showed that on average 67 patients with haematological malignancies on induction chemotherapy are seen. Similar size has been in other related studies e.g. Meidani *et al*., 2013, Kim *et al*., 2011, Massaro *et al*., 2015, Ryu J *et al*., 2015, and Povoa *et al*., 2011 who used an average sample size of 66.

The sample size was then adjusted to take into consideration the population of patients available. The value of N is adjusted after the formula above was been calculated to take into consideration the population of patients available.

Adj sample size = $n/\{1+(n-1/Pop)\}$

Where adj sample size= the adjusted sample size

Pop = Population

n=originally calculated sample size

adj sample size = $\frac{1984}{\{1+ (1984-1/69)\}} = \frac{1984}{29.74} = 66.71 = 67$

Therefore sample size is **67**

3.7. Eligibility criteria

3.7.1 Inclusion criteria

- All cases of haematological malignancies, including leukaemia, lymphomas
- Receiving chemotherapy for malignancy.
- Patient with neutropenia with or without fever
- Willingness to participate in the study after informed written consent.

3.7.2 Exclusion criteria

- Patients who have had a recent major surgery or burns
- Patients with myocardial infarction

3.9. Data collection

3.8.1. Clinical and Demographic Data Collection

Participants were recruited from the paediatric oncology ward and from the adult general wards under the department of medicine of the University Teaching Hospital as well as the Cancer Diseases Hospital during normal clinic hours from 07:00 AM to 12:00 PM, from Monday to Friday. Patient selection was done in collaboration with the attending clinician. As the participants were seen by the clinician, they were informed about the study and also provided with the study information. If a patient or guardian agreed to be part of the study, they were required to sign the consent form. For minors aged sixteen years and below, the minor assent form was used.

Demographic data including the participants age and sex was gathered. The medical history data included the specific type of malignancy a patient has, type of chemotherapy they are taking, any other medication they had taken and the presence of medical conditions that may confound the research findings; included here are any major surgeries, burns, myocardial infarction etc. The patient's files were also reviewed to find any further relevant data to the research and also to confirm the accuracy of information provided by the participant and recorded.

3.8.2. Skin preparation and Specimen Collection

Before collection of blood, the skin was cleansed vigorously over the veni-puncture site in a circle approximately 5cm in diameter with an alcohol swab for about 30 seconds. Starting from the centre of the circle, 10% povidine iodine (betadine) was applied in ever widening circle until the entire circle was saturated with iodine, then left to dry for about 30 to 40 seconds. When collection was complete the iodine was removed from the skin with an alcohol swab. The cap was then removed and the septum disinfected with an alcohol swab and allowed to air dry.

Blood cultures were collected first using a 10 ml 21G syringe for adults and a 5 ml syringe for children. About 8mls of adult blood was then transferred into the blood culture bottle for adults and 1 to 3ml of blood into the paediatric blood culture bottles. The remaining 2mls of blood was transferred into plain containers and labelled appropriately. The blood specimens were

transported to the laboratory within 1 hour after collection for preparation and storage. Blood culture bottles were incubated in the BACTEC™ FX blood culture system immediately upon arrival in the laboratory.

3.8.3. Specimen Preparation and Storage

In the laboratory, the blood specimen in the plain container was centrifuged at 2500 revolutions per minute for three minutes in order to separate the plasma from the blood cellular component. Plasma was then collected from the plain vacutainers using pipettes and transferred to 2ml plastic cryo-vial containers with sealable screw caps and stored in a freezer at - 80°C that is if a delay in specimen analysis was anticipated.

3.9. Quality control

To ensure reliable results, quality control was performed on all the analytical instruments and analysers to be used for any purpose during specimen analysis according to the UTH quality control guidelines. Quality control included regular equipment performance checks of the BACTEC FX system and control runs on the prepared media for purity and growth of control organisms before each test analysis.

3.10 Specimen analysis

3.10.1. Reagent Preparation

All kit components and samples were brought to room temperature before use. The micro-plate (Neoplate) was brought to room temperature before opening. 40mL of wash solution concentrate was diluted 25 times with 960 of distilled water.

3.10.2 Human PCT ELISA KIT

This Human procalcitonin ELISA (Enzyme-Linked Immuno-sorbent Assay) is a quantitative competitive immunoassay. It is manufactured and supplied by Neoscientific group, United States of America. The Neo-plate provided is coated with a PCT specific antibody. Standards or experimental samples are co-incubated in wells along with a PCT conjugate. PCT in standards or samples competes with PCT-HRP (Horseradish Peroxidise) conjugate for binding to the plate bound antibody. Higher levels of PCT from standards or samples leads to decreased PCT-HRP conjugate binding and reduced signal. Captured PCT-HRP is quantitatively detected by incubation with HRP substrates (solutions A and B). Binding of the PCT-HRP is visualized by production of colorimetric reaction products that can be quantitatively measured by absorbance at 450nm. The sensitivity of this assay should be approximately 0.1ng/ml. This assay has high sensitivity and excellent specificity for the detection of PCT. No significant cross-reactivity or interference between PCT and any homologous proteins assayed has been observed.

3.10.3 Human CRP ELISA KIT

This Human procalcitonin ELISA (Enzyme-Linked Immuno-sorbent Assay) is a quantitative competitive immunoassay. It is manufactured and supplied by Neoscientific group, United States of America. The Neo-plate provided is coated with a CRP specific antibody. Standards or experimental samples are co-incubated in wells along with a CRP conjugate. CRP in standards or samples competes with CRP-HRP (Horseradish Peroxidise) conjugate for binding to the plate bound antibody. Higher levels of CRP from standards or samples leads to decreased CRP-HRP conjugate binding and reduced signal. Captured CRP-HRP is quantitatively detected by incubation with HRP substrates (solutions A and B). Binding of the CRP-HRP is visualized by production of colorimetric reaction products that can be quantitatively measured by absorbance at 450nm. The assay has a minimum detectable (sensitivity) dose of C-reactive protein of less than 2pg/ml.

3.10.4 Assay procedure (PCT and CRP)

100µL of sample and standard was added appropriate number of wells in the supplied neo-plate and 100 µL of PBS (pH 7.0 – 7.2) to the blank well. Then 50µL of enzyme solution was added to each well except the blank in the neo-plate and mixed well. The plate was covered and incubated for 1 hour at 37˚C in a humid chamber. Each well was washed 5 times with 300-400µL, 1X wash per well. After the last wash the plate was inverted and dry blot by tapping on absorbent paper. 50 µL of substrate A was added to each well followed by addition of 50µL. The plate was again covered and incubated for 10 to 15 minutes at room temperature out of direct sunlight. Then 50 µL of stop solution was added to each well and mixed well and immediately read the optical density (O.D) at 450nm.

3.9.5. Data Processing

The O.D. of other non-zero standards were divided by that of the zero standards, and then multiplied by 100 (used as X variables). Then, the base 10 logarithm of other standard concentration was calculated (taken as Y variables). A standard curve was generated from these variables in Microsoft Excel 2011 for Mac.

3.10.6. Blood culture (BC) procedure

The BACTEC™ FX blood culture system a fully automated microbiology growth and detection system designed to detect microbial growth from blood specimens was employed in this study. The blood culture instrument offers performance and reliability in the diagnosis of bacteraemia and fungemia. When no bacterial growth is detected by the BD BACTEC machine until five days after primary inoculation of the blood sample, the result of the blood culture was considered negative.

3.10.7. Microbiological analysis

Identification of the organisms that have flagged positive from the BACTEC™ FX blood culture system was performed as follows

Subculture and isolation of the organisms on Blood Agar, Chocolate Agar and MacConkey Agar was done. On the first day, the samples were inoculated on the three different media and incubated between 35°C - 37°C, for 18-24 hours. On the second day, the plates were examined for growth of the organisms. If there was growth, we proceeded with identifying and sub-culturing on fresh media for purity of the organisms. If there was no growth, the plates were re-incubated for another 24 hours. On the third day, from the purified isolates gram stain was performed and organisms identified using the VITEK system.

4.0. STATISTICAL ANALYSIS

Data was analysed with Statistical Package for Social Sciences (SPSS) version 21 and STATA version 12, results were summarised on to tables and graphs. All statistical tests were performed at 5% significance level or 95% confidence interval with p-value of less than 0.05 to determine statistical significance.

Patients were grouped as blood culture positive (bacteraemia group) and blood culture negative (non bacteraemia group) then compared with demographic and clinical characteristics as well as the levels of PCT and CRP in both groups. Descriptive statistics were reported in terms of absolute frequencies and percentages. Distributions of data were described in terms of median value and range, because of the non-normal distribution of CRP values. Accordingly, comparison between groups was performed using the non parametric Mann–Whitney U test. PCT values were normally distributed and were described in terms of mean and standard deviation (SD) values, comparisons between groups were performed using an independent t-test. Data were calculated for sensitivity and specificity, derived from receiver operating characteristics (ROC) curve with blood culture as a reference. Performance indicators of PCT and CRP for various cut-off values were analysed.

5.0. ETHICAL CONSIDERATIONS AND AUTHORISATION

The research proposal for this study was submitted for ethical approval and was approved by the Zambia Biomedical Research Ethics Committee (UNZA BREC) ASSURANCE NO. FWA00000338 IRB00001131 of IORG0000774 (REF No.005-11-15)

Permission to conduct the study was obtained from the UTH and CDH medical superintendent. Patient information and results were confidential and access to this information was restricted to the researcher, supervisor and clinicians only. The data collection booklet captured the participants' file number, which was assigned a serial number hence specimen tubes were identified by serial numbers.

The study participants were provided with an information sheet and given a thorough explanation of intent and rationale of the research after which the patient gave written informed consent without duress, thus ensuring a true meeting of minds between the researcher and the patient. All this was done on a private one to one basis to avoid undue influence that may affect or substitute the patient's will for others. Some of the ethical issues related to this kind of study were taken into consideration included performing a study among children, patients requiring blood transfusion, very sick patients from whom consent cannot be obtained.

6.0 RESULTS

6.1 Study participant characteristics

The distribution and description of study participant's characteristics such as sex, age, presence of fever, levels of the biomarkers and underlying malignancy were analyzed in relation to bacteraemia **(table 01 below).** The median (range) age was 14 (4-43) years in the group with bacteraemia and 16 (4-70) in the group without bacteraemia. A total of 68 participants were enrolled in the study, of which 36 (52.9%) were males and 32 (47.1%) were females. Twenty two (22) of the participants presented with fever and neutropenia while 46 of them only had neutropenia. Bacteraemia was detected in 14 (20.6%) of the 68 patients using blood culture. All patients were suffering from a haematological malignancy and were receiving chemotherapy. Amongst the diagnoses were acute leukaemia (26.5%), Chronic leukaemia (16.2%), Hodgkin's Lymphoma (13.2%), Non-Hodgkin Lymphoma (36.8%), Multiple myeloma (4.4%), Langerhan histiocytosis (2.9%).

Table 04, shows the organisms isolated in patients with haematological malignancies. Fourteen (14) blood cultures were positive out of the total sixty eight (68) patients enrolled in the study among patients with haematological malignancies and gram-positive organisms were the most frequent isolated agents responsible for 71% (10) of positive bacterial cultures, with gram-negative representing 29% (4). Among the gram-positive organisms, the most frequent microbial agents isolated were *staphylococcus spp, bacillus spp, Corynebacteria spp.*

Table 1: Comparison of study participant characteristics between patients with and without bacterial infection

Characteristic	All	Bacteraemia Present	No Bacteraemia present	P-Value
Number (%)	68	14 (20.6)	54 (79.4)	
Age (Median, range)	4-70	14 (4-43)	16 (4-70)	0.169[m]
Sex				
Males	36	7 (50%)	29 (53.7%)	0.805[c]
Females	32	7 (50%)	25 (46.3%)	
Underlying malignancy				
Leukaemia	29	8(57.1%)	21(39.6%)	0.554[f]
Lymphoma	34	6(42.9%)	28(52.8%)	
Multiple myeloma	3	0(0%)	3(5.7%)	
Langerhan histiocytosis	2	0(0%)	2(1.9%)	
Fever (≥38.0˚C)				
No	46	5 (35.7%)	41 (75.9%)	0.004[c]
Yes	22	9 (64.3%)	13 (24.1%)	
Biomarker				
CRP (media, range)		23.5 (6.03-75.44)mg/ml	24.2 (6.43-48.15)mg/ml	0.832[m]
PCT(Mean, SD)		6.1 ± 1.3ng/ml	5.1 ± 1.7ng/ml	0.023[t]

m = mann-whitney test used; c= chi-square; t= independent t test; f= fishers exact test reported r=range

As shown in the table above, all characteristics were not associated with bacteraemia except for PCT levels and presence of fever.

6.2 The distribution of PCT and CRP level between groups

6.2.1 Median CRP Concentration differences

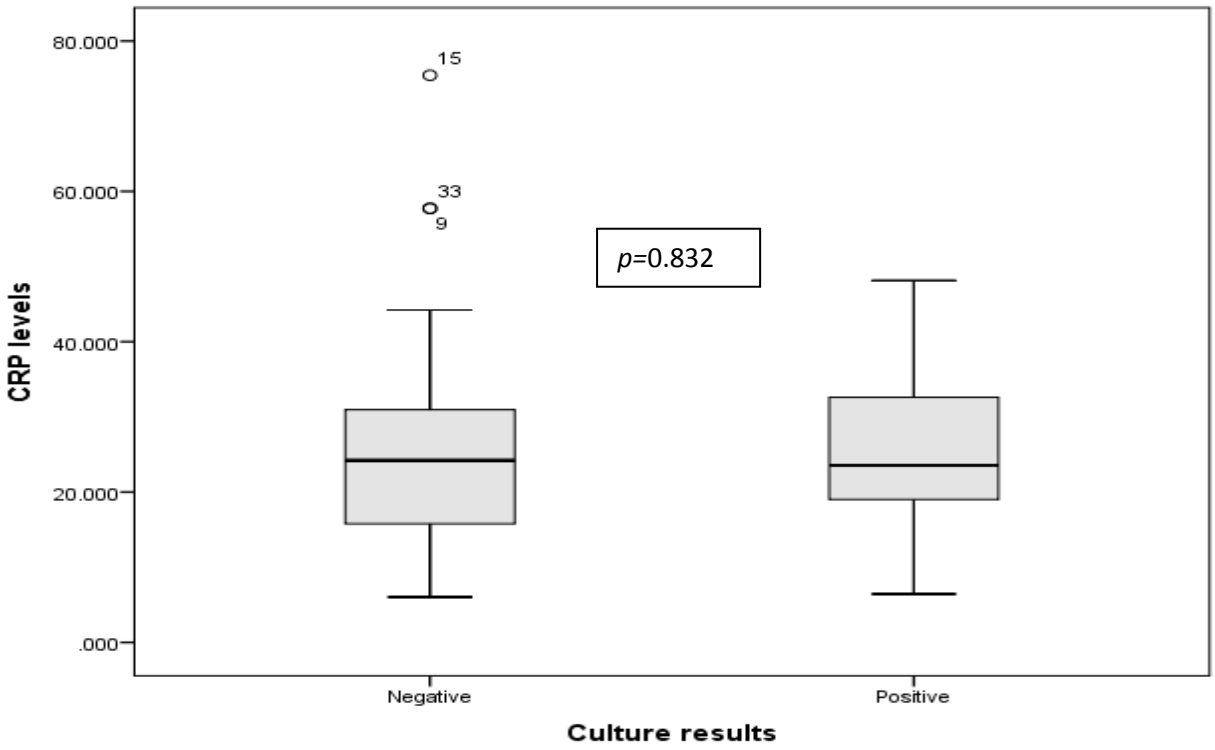

Figure 2, the box plot showing distribution CRP levels for patients with bacteraemia and those without bacteraemia

As shown in **Figure 2**, the median CRP concentrations for the bacteraemia group and non-bacteraemia group were 23.5 (6.03-75.44) mg/ml and 24.2 (6.43-48.15) mg/ml respectively (p=0.832) among patients with haematological malignancies.

6.2.2 Mean PCT Concentration differences

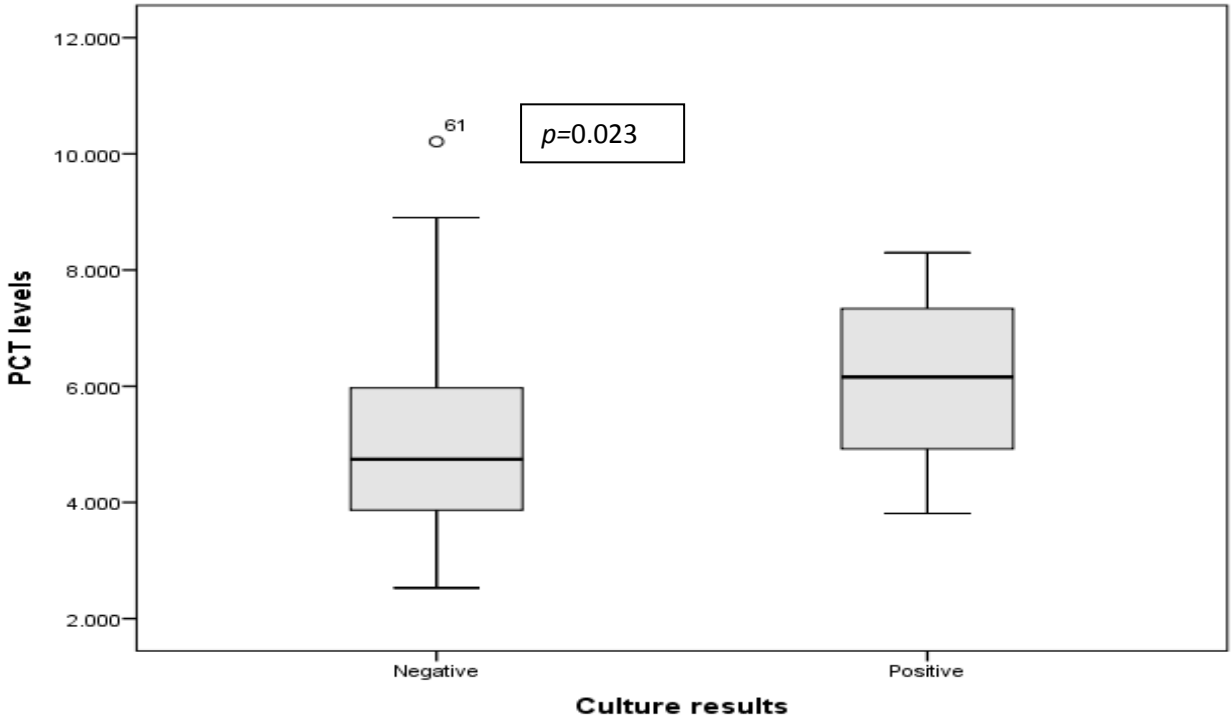

Figure 03, a box plot showing the distribution of PCT levels for patients with and without bacteraemia. The mean PCT concentrations for the group with bacteraemia and those without were 6.1 ± 1.3ng/ml and 5.1 ± 1.7ng/ml respectively. As shown, the bacteraemia group had higher mean PCT levels than those without bacteraemia (p=0.023).

6.3 Diagnostic accuracy for predicting bacteraemia

6.3.1 Area under ROC curve for PCT and CRP

Receiver operating Curves (ROC) were used to determine the diagnostic value of using CRP and PCT to predict a bacterial infection in patients with haematological malignancies **(Figure 04)**. As shown in **table 02,** the area under curve (AUCs) was 0.702 (95% CI =0.35 - 0.69) for PCT and 0.519 (95% CI=0.57 – 0.84) for CRP. Area under curves values for each marker PCT (0.702) demonstrates greater discriminatory ability than CRP (0.52) to differentiate infections. The results also showed that the AUC for PCT (0.702) was statistically significant in discriminating between bacteraemia and no bacteraemia (p=0.021), compared with CRP (p=0.832)

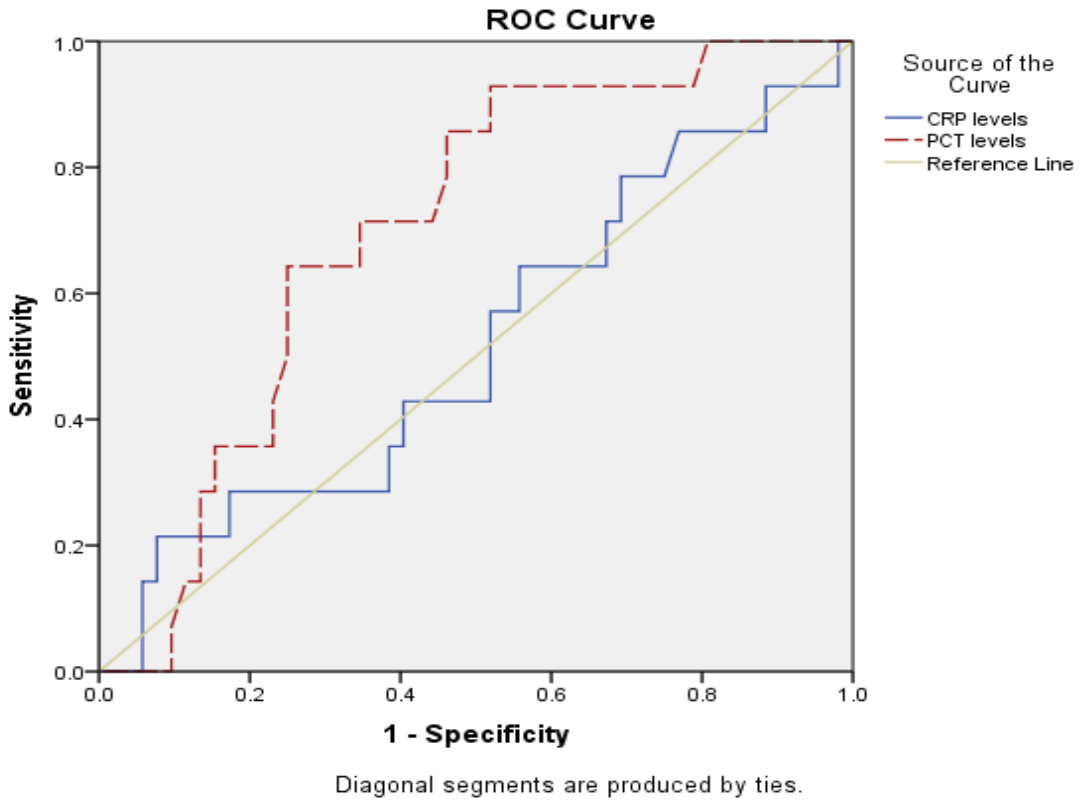

Figure 04, the receiver operating characteristic curves for procalcitonin and C-reactive protein levels with blood culture results as a reference.

Table 02, the table shows the areas under the ROC curves of PCT and CRP (70% and 52%, respectively), and the Confidence intervals (CI).

Biomarker	AREA	Standard error	95% CI	P-Value
PCT	0.702	0.069	0.345 – 0.693	0.021
CRP	0.519	0.089	0.566 – 0.838	0.832

As can be seen from the table, the AUC for PCT (p=0.021) was significant compared with CRP in predicting a bacterial infection.

6.3.2 Performance indicators at different Cut-off values.

From **Table 3**, Cut offs for PCT that we used were 4.7ng/ml, 5.0ng/ml, 5.1ng/ml. At a cut off value 5.0ng/ml we found diagnostic sensitivity and specificity of 71% and 61% respectively. When the cut off was 4.7ng/ml, the sensitivity and specificity obtained were 86% and 54 respectively while at the cut off of 5.1 showed diagnostic sensitivity and specificity of 71.4% and 65.4%. These cut-off values were chosen based on the best diagnostic sensitivity and specificity obtained from ROC curve analysis and according to literature. The cut offs for CRP were not satisfactory as both the diagnostic sensitivity and specificity were low but a cut off of 21mg/ml gave us the sensitivity and specificity of 64% and 44% respectively.

Table 3, the table shows diagnostic sensitivity and specificity, as well as positive and negative likelihood ratios for PCT and CRP at different cut offs.

Markers	Cut offs	Diagnostic Sensitivity (%)	Diagnostic Specificity (%)	LR+	LR-
PCT	5.0	71.4	61.5	1.85	0.47
	5.1	71.4	65.4	2.06	0.44
	4.7	86	54	1.87	0.26
CRP	20.88	64.3	42.3	1.11	0 .84
	21.0	64.3	44.2	1.15	0.64
	23.5	50	48	0.96	1.04

Legend: Positive likelihood ratio (LR+), negative likelihood ratio (LR-)

The best cut off for PCT is 5.1 with the diagnostic sensitivity and specificity of 71% and 65% respectively. At this cut off the likelihood ratios indicate small to moderate increase (LR+) and decrease (LR-) in the likelihood of the disease which were acceptable compared to other cut-off values.

6.3.3 Correlations of PCT and CRP in patients with haematological malignancies

Spearman correlation coefficient indicated a positive correlation (r=0.39, p < 0.001) between levels of PCT and CRP, as can be seen in **Figure 5**, in both groups with and without bacteraemia. This shows a good relationship or correlation between the two biomarkers.

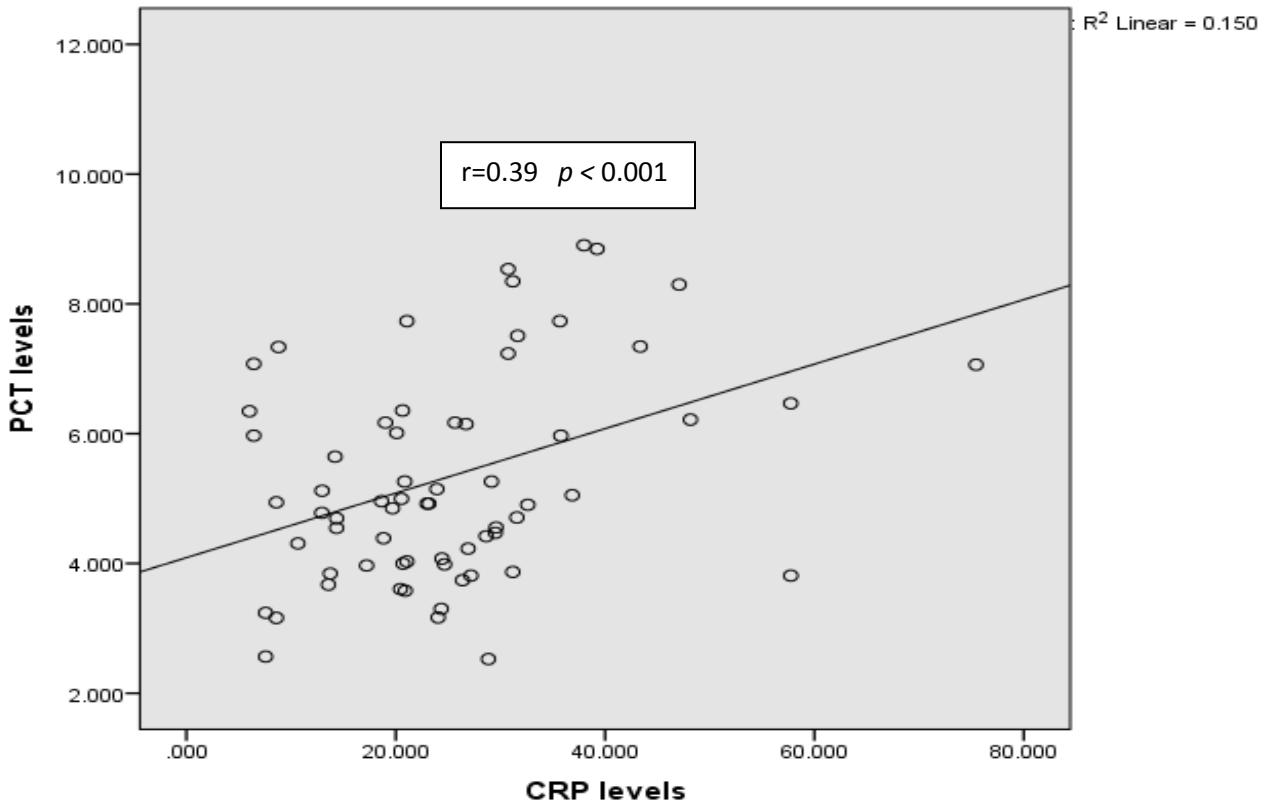

Figure 05, the correlation between the concentrations of C-reactive protein (CRP) and procalcitonin (PCT)

6.4 Bacterial infections isolated

Fourteen (14) blood cultures were positive out of the total sixty eight (68) patients enrolled in the study among patients with haematological malignancies and gram-positive organisms were the most frequent isolated agents responsible for 71% (10) of positive cultures, with gram-negative representing 29% (4), as shown in **table 4**.

ORGANISM		**NO. OF ISOLATED**
Gram Positive Bacteria (GPB)		
Coagulase Negative Staphylococcus	-*Staphylococcus hominis*	04
	-*Staphylococcus epidermidis x2*	
	-*Staphylococcus capitis*	
Staphylococcus aureus		02
Corynebacteria jeikeium		02
Bacillus spp		02
Gram Negative Bacteria (GNB)		
Klebsiela pneumonia		01
Escherichia coli		02
Enterobacter cloacae		01
TOTAL		14

Table 4, bacterial infections isolated from patients with haematological malignancies.

As can be seen from the table, a higher distribution of gram positive bacteria has been noted in this study.

7.0 DISCUSION

Patients with haematological malignancies are immunocompromised due to the neoplastic process and chemotherapy, and hence, are susceptible to frequent infections. Early markers to detect infections are needed especially in neutropenic cancer patients that are produced independent of the leukocyte count and the activity of the underlying disease. The value of the inflammatory biomarkers such as PCT and CRP, in the diagnosis and assessment of infection in non-neutropenic patients has been well established in clinical studies during the past decade (Fleischhack et al., 2000).

Although it has been shown that PCT can also be produced by leukocytes (Oberhoffer et al, 1999), Carnino *et al* (2010) reported that PCT and CRP react more, in deeply neutropenic patients. More studies involving immunocompromised patients have recently demonstrated that these patients are also capable of producing high PCT levels in severe systemic bacterial infections (Ruokonen *et al*., 1999; Sauer et al, 2000; Kim *et al*., 2011). Our results support these findings, despite concerns that these markers may be produced in low concentration in patients with neutropenia (Kim *et al*., 2011). Most studies have demonstrated a significant increase of PCT levels in patients with severe bacterial infection and/or sepsis in non-neutropenic patients (Dipalo *et al*., 2014), fewer data is available that support the use of PCT and CRP in neutropenic cancer patient patients as an indicator of bacteraemia.

Chemotherapy suppresses the hematopoietic system, impairing host protective mechanisms, resulting in neutropenia. This predisposes patients with cancer to infections both by suppressing the production of neutrophils and by cytotoxic effects on the cells that line the alimentary tract. Neutrophils are the first line of defense against infection as the first cellular component of the inflammatory response and a key component of innate immunity. Neutropenia blunts the inflammatory response to nascent infections, allowing bacterial multiplication and invasion (Crawford *et al*., 2004).

7.1 CRP and PCT Concentration differences between groups

The mean PCT levels were statistically different in patients with bacteraemia and the group (p=0.023), with PCT being more elevated among patients with bacteraemia. This was not observed with CRP levels between the two groups as there was no significant differences between the two groups (p=0.832). These findings were similar to a study done by Marshal *et al*, where they found that PCT levels were higher (p< 0.01) in bacteraemia [21] but CRP showed no statistical significance (p=0.79). Our study demonstrated that PCT levels increased significantly in the presence of bacteraemia.

The source of PCT is not well known as production of PCT during an infection is not related to thyroid tissues (Russwurm *et al*., 1999), which is the primary site of production in normal healthy individuals. The exact site of production during an infection has not been identified, but it has been demonstrated that immunoreactive cells including neutrophils where possible sources of PCT production (Kim *et al.*, 2011). However, production of CRP is fairly well understood and it is believed to be produced by the liver in response to IL-6 during an infection or inflammatory states (WHO, 2014).

7.2 Diagnostic performance in predicting bacteraemia

The area under the ROC curve for PCT was found to be 0.702 in contrast to 0.519 for CRP for discriminating between bacteraemia and no bacteraemia. It can be seen from these findings that CRP has poor discriminatory power and hence cannot be reliably used as a diagnostic marker of bacteraemia in patients with haematological malignancies receiving chemotherapy. These results are comparable to the results of Massaro *et al.*, (2007) where AUC for PCT was 0.79 and 0.50 for CRP for diagnosing systemic infection in febrile neutropenic patients.

The cut offs for CRP were not satisfactory as both the sensitivity and specificity were low. The best cut off from our analysis was 21mg/ml which gave us the diagnostic sensitivity and specificity of 64% and 44% respectively. The best cut off for PCT was 5.1ng/ml which corresponds to the sensitivity and specificity of 71% and 65% respectively with relatively

favourable likelihood ratios as compared to other cut off values. These results imply that PCT is a marker that might help clinicians to detect bacteraemia early in patients with haematological malignancies on chemotherapy. A similar cut off for PCT reported in our study was suggested in another study, where they reported that PCT concentrations of between 1ng/ml to 5ng/mL are strongly suggestive of bacteraemia (Massaro *et al*., 2007).

CRP which is commonly used in clinical practice was shown in this study to be a poor tool for distinguishing bacterial infection, given its low sensitivity and specificity (64% and 44%) respectively. From our results, PCT performed better than CRP for early detection of bacterial infection. High diagnostic sensitivity is an essential quality for a marker in order to be valuable in the selection of patients who have bacteraemia and in whom antibiotics can safely be withheld.

The characteristics of CRP, such as its insufficient specificity, and vulnerability to immuno-suppressant, are probably reflected by these results. It is also known to be a sensitive marker of inflammation caused by high tumour load, malignant cell lyses, or drug administration as well as infections (Schuttrumpf *et al*., 2006). This could have contributed to it being a poor marker of bacteraemia given our patient population. However, PCT is better at detecting bacteria in blood but not necessarily its absence, since the specificity is not high enough. Therefore, we suggest that the use of this marker should always be followed up with standard microbiological analysis.

Earlier studies have demonstrated the superiority of PCT in the diagnosis of bacteraemia in contrast to CRP among patients with haematological malignancies on chemotherapy. Higher sensitivities and specificities have been demonstrated for PCT in comparison to CRP (Meidani *et al*., 2013; Schttrumpf *et al*., 2006; and Kim *et al*., 2011). Their findings are comparable to results reported in this study. Meta-analysis studies have also shown sensitivity in the range of 60-96% and specificity of 50-85% for PCT (Sakr *et al*., 2008; Simon *et al*., 2004; Xiao *et al*., 2015) and, they noted that PCT was more specific than CRP. Therefore, PCT has been proposed to have a high value in the diagnosis of bacteraemia in patients with neutropenia. Dipalo *et al*., (2014), demonstrated that bacterial infections are capable of triggering ubiquitous expression of the calcitonin gene (CALC-1) and release of PCT from many cell types. Many other studies have

reported the specificity of PCT to bacterial than other causes of infections or inflammation (Fleischhack et al., 2000; Schuttrumpf et al., 2006; Sandri *et al*., 2008). This probably explains the superiority of PCT compared to CRP, in the diagnosis of bacteraemia.

We further explored the relationship between PCT and CRP and found that there was positive correlation ($r=0.39$, $p<0.001$). These findings indicate a close link between the two markers. That is, the variations in PCT concentration were concomitant with the variations in CRP concentrations. Further, studies are required to determine the value of constructing combinations between PCT and CRP, this might yied a higher diagnostic value than examining them individually.

7.3 Microbiological findings

From previous studies, the incidence of documented bacteraemia has been estimated to be between 7.7% and 37% in patients with neutropenic fever (Gencer et al., 2003; Perola et al., 2005). In this study we found the prevalence of bacterial infection in patient with haematological malignancies with neutropenia to be 14 (20.6%) out of 68 patients. We also noted in this study that infections were more common in patients who were less than 16 year of age compared to those who were advanced in age. The immune system of children is still developing hence they are more vulnerable to infection than adults.

In our study, Gram positive bacteria were the most commonly isolated organisms in comparison to Gram negative bacteria. This finding was in line with a study that indicated a shift in the cause of bacteraemia in cancer patients from predominance of gram-negative rods to gram-positive cocci (Ramphal, 2004). During the past two decades gram negative bacteria emerged as the main causative agents, infections with *Pseudomonas aeruginosa* were common and associated with a high mortality rate. Lately, gram-positive microbes have emerged (Ramphal, 2004). The most common infective agent is *Coagulase-negative staphylococci*, mainly *Staphylococcus epidermidis* (Hämäläinen, 2010). The reasons for this shift to gram positive microbes have not been clearly identified but the following have been suggested; long indwelling intravascular

devices, high dose chemotherapy inducing mucositis, more profound and prolonged neutropenia (Zinner, 1999; Ramphal, 2005; Hämäläinen, 2010).

Among gram-positive microbes causing bloodstream infections the most common finding was *Coagulase negative staphylococcus*, as similarly observed in another study (Hämäläinen, 2010). It is important to be aware of the potential of any organism to cause serious infections, even though those organisms previously were thought to be harmless skin commensals (Zinner, 1999). In fact, all the gram positive organisms that were isolated are skin commensals except for *Staphylococcus aureus.* This strongly suggests these gram positive organisms are more of nosocomial infections or this could have been contamination from the skin. There is inherent risk of infection that some low-virulent gram positive organisms pose in immunocompromised patients (Lodahl and Schrøder, 2011). Although, a shift in favour of gram positive organisms infection has been noted among patients with neutropenia, gram negative organisms are still important and are associated with high mortality (Zinner, 1999).

8.0 CONCLUSIONS

From our study procalcitonin was a better indicator of bacteraemia than C-reactive protein. Procalcitonin measurements can be of value in the early diagnosis of bacteraemia in patients with haematological malignancies. In contrast, the diagnostic sensitivity and specificity for CRP were too low to safely rely on it as a marker of bacteraemia in these patients. We found a close association between PCT and CRP as their levels correlated significantly. Our data also showed a higher distribution of gram positive organisms isolated from patients with a predominance of *Coagulase negative staphylococcus* infections and the incidence of bacteraemia at 21% among patients with haematological malignancies receiving chemotherapy.

8.1 Implications and recommendations

The most widely used biomarker in clinical practise as diagnostic indicator of bacterial infection is C-reactive protein (CRP) performed poorly compared to PCT. Therefore, PCT is more useful or valuable in early diagnosis of bacterial infections in patient with haematological malignancies

Considering that the specificity for PCT was not high enough to exclude standard examinations. These biomarkers should not replace a thorough clinical evaluation and microbiological examinations.

Despite its high cost PCT is more valuable than CRP in discriminating between patients with bacteraemia and those without bacteraemia and should be considered for clinical use.

A predominance of gram positive low virulent skin commensal bacteria compared to gram negative bacteria was noted in this study, suggesting more of nosocomial infections than community acquired bacteraemia. Therefore, there is an urgent need for health worker attending to patient with haematological malignancies with neutropenia to strictly adhere to infection prevention guidelines especially among paediatric patients.

8.2 Limitations

A limitation of this study for both PCT and CRP is that these results only reflect systemic manifestation of bacterial infection. We may not obtain similar results when an infection remains in a localized tissue with no systemic manifestations e.g. urinary tract infection (UTI). It has been suggested that serial measurements of PCT and CRP can help to better understand their diagnostic capacity. We could not do this in this study due to resource constraints and limited time. The other limitation related to this study is that other possible causes of infections including viral and fungal infections maybe confounders to our findings.

8.3 Future Direction

PCT remains an interesting marker for further investigation in the neutropenic population since currently; it is not used in the standard of care of patient with neutropenia. Further studies, with larger samples, involving PCT of febrile severely neutropenic patients should be performed in order to confirm our results.

Future studies should explore the value of constructing combinations of PCT and CRP as well as other markers for example IL-6 and IL-8 that can predict an infection. These combinations may improve diagnostic efficiency with higher sensitivity and specificity values than when evaluated individually.

At present, the lack of an appropriate microbiological method applicable in clinical laboratories to get the result early to allow for prompt treatment and the lack of practical cut-off values with a satisfactory diagnostic sensitivity and specificity for these markers still poses a problem for clinicians in the management of these patients and requires further investigation.

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10.0 APPENDICIES

10.1. Parents or guardian Information sheet (for adult patients)

My name is Kamvuma Kingsley, studying for a MSc. Pathology (Haematology) at the University of Zambia. I' am carrying out a research (study) as a requirement for fulfilment of the Master Degree program, please feel free to ask any question as I explain about the study.

About the Study:

The study will seek to determine the levels of two markers (Procalcitonin and C reactive protein) that can be used to detect infections in blood. Patients with cancers of the blood have a weak immune system due to the spread of the cancer and the drugs used to treat it. Timely diagnosis of infectious complications in these patients is difficult, because a severe infection may be asymptomatic or manifest only in the form of fever or malaise.

Considering that obtaining of results of microbiological tests may take a long time, these markers of infection will assist in detecting bacterial infections early enough to allow for prompt initiation of antibiotic therapy.

Participating in this Study:

You are invited to take part in this research study. You can decide whether you want to participate in the study or not. You are free to say yes or no. And even if you join this study, you do not have to stay in it. You may stop at any time without penalty. You will be asked a few questions which you may not answer, if through your own understanding seem personal, uncomfortable or otherwise. You will also be requested to give consent to use blood that is collected from you by the nurse for routine monitoring of the condition. This blood is needed because it will be used to measure the markers mentioned above and for culture.

Benefits for participation:

There are no costs to you for being in this study and the study may not benefit you directly. However the study results will provide valuable information about your health and this information will be made available through your physician. It is hoped that information

generated through this study as a result of your participation will not only help you as a participant but also lead to improved management of patients with this condition in the future. Thus your participation in this study may help thousands of people in many years to come.

Risks of the study:

The risks that some people have experienced in studies like this are some pain when blood is being drawn from the vein or loss of confidentiality. Every effort will be made to reduce the pain that you feel as the blood is collected. It is also important to understand that obtaining a blood sample from some of the participants may be more difficult than from others.

The Chairperson
Research Ethics Committee
P.O .Box 50110
Lusaka
Contact Number:256067

10.2 Parent/guardian information sheet (for minors)

Project Title: **Procalcitonin and C-reactive protein as early markers of infections among patients with haematological malignancies receiving chemotherapy**

Investigator: **KAMVUMA M KINGSLEY**

We are doing a research study about patients with haematological cancers. These patients have a weak immune system because of the cancer and the medicines they are given, making them to have infections frequently. The study will try to find out whether markers (i.e. PCT CRP) can be used to detect these infections early to allow for prompt treatment.

If you decide that you want to be part of this study, we will request for information about you such as your age, sex, drugs you are taking, how long you have had the malignancy, and whether you are taking antibiotics or not. You also need to know that when you accept to participate in the study we will collect blood from you which may cause you minor discomfort.

There are no benefits related to taking part in this study but information that will generated as a result of your participation may help other patients that will develop a condition like yours to be managed properly.

If you do not want to be part of this research study, you will not be included in the study. Even if you accept to be part of the study you are free at any time to withdraw from the study. We will also request your parents to allow us include you in the study.

When we are finished with this study we will write a report about what was learned. This report will not include your name or that you were in the study. If you decide you want to be in this study, you will be required to sign the participant informed consent form

The Chairperson

Research Ethics Committee

P.O .Box 50110
Lusaka
Contact Number: 256067.

10.3 Participant informed consent form for the study

APPENDIX II: INFORMED CONSENT FORM

STUDY TITLE:

Procalcitonin and C-reactive protein as early markers of infections among patients with haematological malignancies receiving chemotherapy

By signing my name below, I ……..…….. Confirm the following:

- I have read (or had read to me) this entire consent document and all of my questions have been answered adequately.

- The study's purpose, procedures, risks and possible benefits have been explained to me.
- I freely and voluntarily choose to participate.
- I understand that participating or not will not affect my health care or that of my family members.
- I understand that my rights and privacy will be maintained.

Participant signature …… Date……..

Thumb print if participant can't sign…….

……. …….

Witness (Name and Signature) Date

Declaration by member of research team/taking consent:

I have given a verbal explanation of the research project to the participant, and have answered the participant's questions about it to the best of my ability.

I believe that the participant understands the study and has given informed consent to participate**.**

Researcher's Name:

Signature: **Date:**

NOTE: *The participant will be provided with a signed and dated copy of this consent form. It will help him remember what we discussed today.*

Contact Details

In case you have any more questions about this study at any time, please feel free to contact any of the numbers below.

1. **Kamvuma M Kingsley**
   The Principal Investigator
   Contact Number: +260 978 083356
   Email Address: kamvumak@yahoo.com

2. **Dr S.K. Kowa**
   Principle Supervisor
   Contact Number: +260955920473
   E-Mail Address:kwxsum001@myuct.ac.za

3. **The Chairperson**
   Research Ethics Committee
   P.O .Box 50110
   Lusaka
   Contact Number:02112560

10.4 Participants Raw Data

IDs	SEX	AGE	FEVER PRESENT	CULTURE	CRP	PCT	UNDERLYING MALIGNANCY	GRAM STAIN	ORGANISM ISOLATED
H01	Male	42	No	Negative	8.589	3.161	Lymphoma	No growth	
H02	Male	5	Yes	Negative	37.981	8.903	lymphoma	No growth	
H03	Female	4	No	Negative	6.025	6.346	Leukemia	No growth	
H04	Male	46	No	Negative	7.567	2.564	Leukemia	No growth	
H05	Male	6	No	Negative	20.444	3.606	Lymphoma	No growth	
H06	Male	14	No	Negative	6.430	5.970	Lymphoma	No growth	
H07	Male	15	Yes	Positive	14.348	4.695	Leukemia	GNB	*E. cloacae*
H08	Male	5	Yes	Negative	39.232	8.847	Lymphoma	No growth	
H09	Female	38	Yes	Negative	57.734	6.467	Lymphoma	No growth	
H10	Female	17	No	Negative	17.215	3.968	Lymphoma	No growth	
H11	Female	21	Yes	Negative	30.745	8.535	Lymphoma	No growth	
H12	Male	28	No	Negative	14.348	4.547	Leukemia	No growth	
H13	Female	6	Yes	Positive	8.804	7.335	Leukemia	GNB	*E .coli*
H14	Male	26	Yes	Negative	75.444	7.063	Leukemia	No growth	
H15	Female	43	Yes	Negative	31.181	8.347	Lymphoma	No growth	
H16	Female	30	No	Negative	12.967	5.121	Leukemia	No growth	
H17	Female	5	No	Negative	20.691	3.998	Lymphoma	No growth	
H18	Male	9	No	Negative	13.742	3.844	Lymphoma	No growth	
H19	Female	12	No	Positive	23.928	5.146	Leukemia	GPB	*C. jeikieum*
H20	Female	35	No	Negative	21.066	4.030	Leukemia	No growth	
H21	Male	15	No	Negative	24.350	3.300	Leukemia	No growth	
H22	Male	10	No	Negative	29.587	4.554	Leukemia	No growth	
H23	Female	16	No	Negative	26.376	3.742	Leukemia	No growth	
H24	Female	13	No	Negative	8.589	4.941	Lymphoma	No growth	
H25	Male	47	No	Negative	31.180	3.869	Leukemia	No growth	
H26	Male	15	No	Negative	13.573	3.674	Lymphoma	No growth	
H27	Male	9	No	Negative	24.411	4.075	Leukemia	No growth	

H28	Female	45	No	Negative	29.513	4.471	Lymphoma	No growth	
H29	Female	9	No	Negative	12.944	4.779	Leukemia	No growth	
H30	Male	15	No	Positive	27.178	3.812	Leukemia	GPB	*S.epidermidis*
H31	Male	5	No	Positive	23.163	4.922	Leukemia	GPB	*Bacilli spp*
H32	Female	18	No	Negative		3.297	Leukemia	No growth	
H33	Male	25	No	Negative	57.743	3.812	Lymphoma	No growth	
H34	Male	18	Yes	Negative	35.682	7.734	Lymphoma	No growth	
H35	Female	14	No	Negative	20.909	3.580	Lymphoma	No growth	
H36	Male	7	No	Positive	47.082	8.298	Lymphoma	GPB	*C. jeikieum*
H37	Female	6	No	Negative	20.547	4.996	Lymphoma	No growth	
H38	Male	44	No	Negative	28.857	2.527	Lymphoma	No growth	
H39	Male	55	No	Negative	22.991	4.922	Lymphoma	No growth	
H40	Male	56	No	Negative	18.827	4.389	Lymphoma	No growth	
H41	Female	41	No	Negative	24.048	3.168	Lymphoma	No growth	
H42	Female	4	Yes	Positive	20.090	6.012	Leukemia	GNB	*E. coli*
H43	Female	11	No	Negative	31.651	7.508	Leukemia	No growth	
H44	Female	4	No	Positive	6.430	7.076	Leukemia	GPB	*S. epidermidis*
H45	Female	70	No	Negative	31.572	4.708	Lymphoma	No growth	
H46	Male	46	Yes	Negative	7.567	3.239	Leukemia	No growth	
H47	Male	15	Yes	Negative	36.860	5.052	Leukemia	No growth	
H48	Female	14	No	Negative	24.656	3.986	Lymphoma	No growth	
H49	Male	4	No	Negative	30.744	7.235	Lymphoma	No growth	
H50	Female	8	No	Negative	18.640	4.959	Leukemia	No growth	
H51	Male	6	No	Negative	25.661	6.170	Leukemia	No growth	
H52	Male	15	Yes	Positive	43.357	7.343	Lymphoma	GPB	*S. aureus*
H53	Male	9	Yes	Negative	28.642	4.421	Multiple myeloma	GPB	*Bacillus spp*
H54	Female	27	Yes	Negative	26.908	4.229	Lymphoma	No growth	
H55	Female	14	No	Negative	35.771	5.968	Lymphoma	No growth	

H56	Male	10	Yes	Positive	32.614	4.904	Lymphoma	GPB	*S. capitis*
H57	Female	25	No	Negative	29.147	5.262	Leukemia	No growth	
H58	Female	5	Yes	Negative	26.707	6.147	Lymphoma	No growth	
H59	Female	65	Yes	Negative	10.653	4.308	Multiple myeloma	No growth	
H60	Male	8	No	Negative	20.857	5.262	Leukemia	No growth	
H61	Female	30	No	Positive	44.232	10.20	lymphoma	No growth	
H62	Male	5	No	Negative	14.197	5.645	Lymphoma	No growth	
H63	Male	4	Yes	Negative	20.649	6.356	Leukemia	No growth	
H64	Male	15	Yes	Positive	21.066	7.734	Leukemia	GPB	*S. hominis*
H66	Female	43	Yes	Positive	48.152	6.216	lymphoma	GPB	*S. aureus*
H67	Female	39	Yes	Positive	19.016	6.170	lymphoma	GNB	*K. pneunomiae*
H65	Male	34	No	Negative		5.024	Leukemia	No growth	
H68	Male	12	No	Negative	19.692	4.850	lymphoma	No growth	

THE UNIVERSITY OF ZAMBIA

SCHOOL OF MEDICINE

Telephone : +260211252641
Telegram: UNZA, Lusaka
Telex: UNZALU ZA 44370

P.O Box 50110
Lusaka, Zambia

Email: assistantdeanpgmedicine@unza.zm

15th October, 2015

Mr. Kingsley Kamvuma
Department of Pathology & Microbiology
School of Medicine
UNZA
LUSAKA

Dear Mr. Kamvuma,

RE: GRADUATE PROPOSAL PRESENTATION FORUM

Following the presentation of your dissertation entitled **"Procalcitonin and C – Reactive Protein as early as markers of Infection among Patients with Haematological Malignancies receiving Chemotherapy at the University of Teaching Hospital "** your supervisor has confirmed that the necessary corrections to your research proposal have been done.

You can proceed and present to the Research Ethics.

Yours faithfully,

Dr. S.H. Nzala
ASSISTANT DEAN, POSTGRADUATE
Cc: HOD, Pathology & Microbiology

UNIVERSITY OF ZAMBIA
SCHOOL OF MEDICINE
15 OCT 2015
ASSISTANT DEAN (PG)
P.O. BOX 50110, LUSAKA

All Correspondence should be addressed to the
Executive Director
Tel/Fax: +260 211 257706

In reply please quote

No. CDH/101/1/13

REPUBLIC OF ZAMBIA
MINISTRY OF HEALTH
CANCER DISEASES HOSPITAL

P. O. Box Rw 51337
LUSAKA

November 4, 2015

TO WHOM IT MAY CONCERN

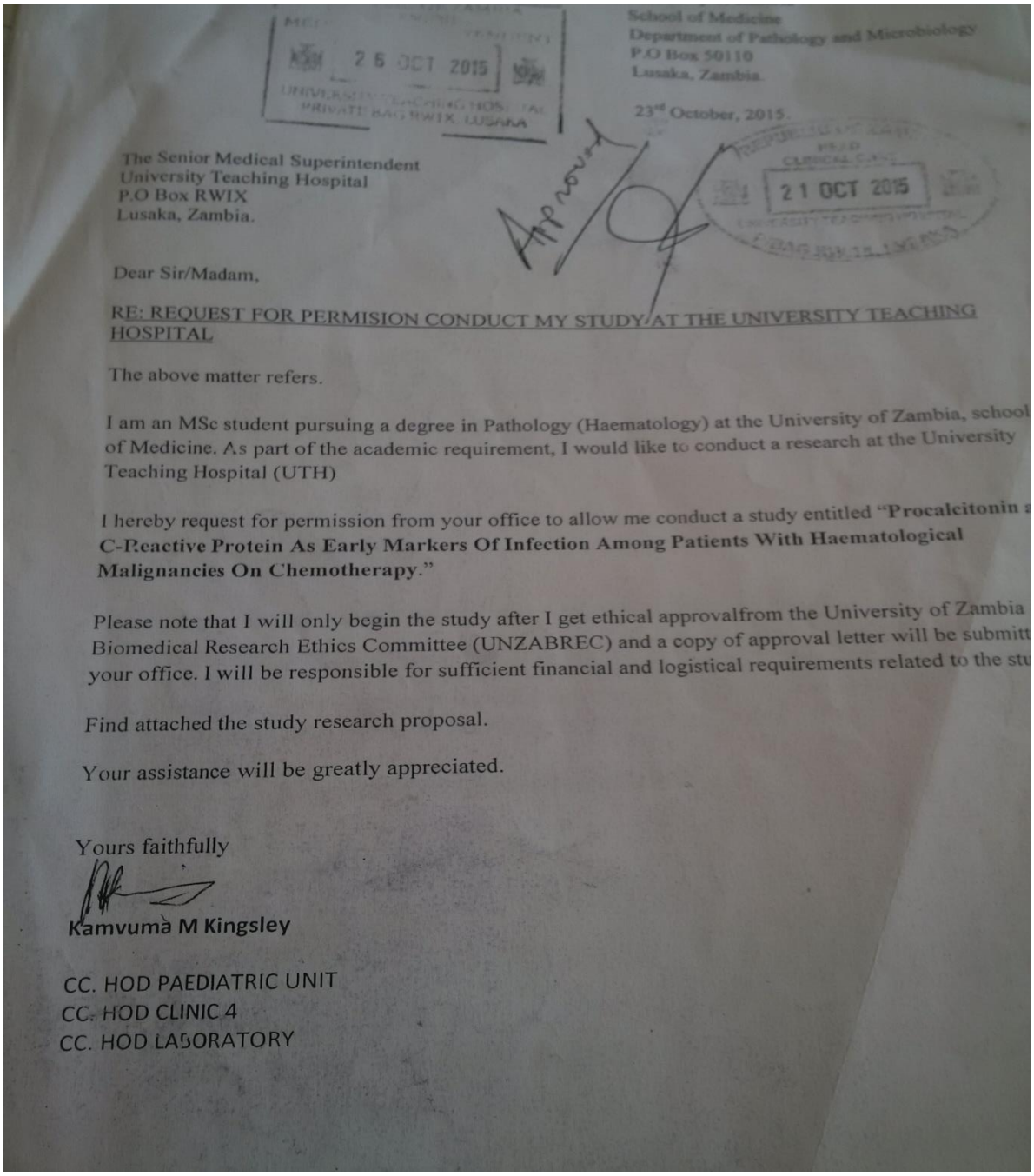
Dear Sir/ Madam,

RE: AUTHORITY FOR KAMVUMA M. KINGSLEY TO CONDUCT A RESEARCH AT THE CANCER DISEASES HOSPITAL

The Cancer Diseases Hospital would like to inform you that we have granted permission to Kamvuma M Kingsley to conduct a research at our hospital for a project proposal titled: **"Procalcitonin and C-Reactive Protein as Early Markers of Infection among Patients with Haematological malignancies Receiving Chemotherapy".**

Yours sincerely

DR. S.C. MSADABWE-CHIKUNI, BSc MB ChB, FC Rad Onc (SA), M.Med Rad Onc (Wits)
ACTING SENIOR MEDICAL SUPERINTENDENT1



26 OCT 2015

UNIVERSITY TEACHING HOSPITAL
PRIVATE BAG RWIX, LUSAKA

School of Medicine
Department of Pathology and Microbiology
P.O Box 50110
Lusaka, Zambia.

23rd October, 2015.

Approved

21 OCT 2015

The Senior Medical Superintendent
University Teaching Hospital
P.O Box RWIX
Lusaka, Zambia.

Dear Sir/Madam,

RE: REQUEST FOR PERMISION CONDUCT MY STUDY AT THE UNIVERSITY TEACHING HOSPITAL

The above matter refers.

I am an MSc student pursuing a degree in Pathology (Haematology) at the University of Zambia, school of Medicine. As part of the academic requirement, I would like to conduct a research at the University Teaching Hospital (UTH)

I hereby request for permission from your office to allow me conduct a study entitled "**Procalcitonin a C-Reactive Protein As Early Markers Of Infection Among Patients With Haematological Malignancies On Chemotherapy**."

Please note that I will only begin the study after I get ethical approvalfrom the University of Zambia Biomedical Research Ethics Committee (UNZABREC) and a copy of approval letter will be submitt your office. I will be responsible for sufficient financial and logistical requirements related to the stu

Find attached the study research proposal.

Your assistance will be greatly appreciated.

Yours faithfully

Kamvumà M Kingsley

CC. HOD PAEDIATRIC UNIT
CC. HOD CLINIC 4
CC. HOD LABORATORY

THE UNIVERSITY OF ZAMBIA

BIOMEDICAL RESEARCH ETHICS COMMITTEE

Telephone: 260-1-256067
Telegrams: UNZA, LUSAKA
Telex: UNZALU ZA 44370
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Ridgeway Campus
P.O. Box 50110
Lusaka, Zambia

Assurance No. FWA00000338
IRB00001131 of IORG0000774

15th February, 2016.

Our Ref: 005-11-15.

Mr. Kingsley M. Kamvuma,
University of Zambia,
School of Medicine,
Department of Pathology and Microbiology,
P.O Box 50110,
Lusaka.

Dear Mr. Kamvuma,

RE: RESUBMITTED RESEARCH PROPOSAL: **"PROCALCITONIN AND C-REACTIVE PROTEIN AS EARLY MARKERS OF INFECTION AMONG PATIENTS WITH HAEMATOLOGICAL MALIGNANCIES RECEIVING CHEMOTHERAPY" (REF. No. 005-11-15)**

The above-mentioned research proposal was presented to the Biomedical Research Ethics Committee on 10th February, 2016. The proposal is approved.

CONDITIONS:

- This approval is based strictly on your submitted proposal. Should there be need for you to modify or change the study design or methodology, you will need to seek clearance from the Research Ethics Committee.
- If you have need for further clarification please consult this office. Please note that it is mandatory that you submit a detailed progress report of your study to this Committee every six months and a final copy of your report at the end of the study.
- Any serious adverse events must be reported at once to this Committee.
- Please note that when your approval expires you may need to request for renewal. The request should be accompanied by a Progress Report (Progress Report Forms can be obtained from the Secretariat).
- **Ensure that a final copy of the results is submitted to this Committee.**

Yours sincerely,

S.H Nzala
VICE-CHAIRPERSON

Date of approval: 15th February, 2016. **Date of expiry:** 14th February, 2017.