MAKERERE UNIVERSITY

HUMAN CYTOMEGALOVIRUS INFECTION IN FEBRILE PATIENTS WITH HEMATOLOGICAL MALIGNANCIES AT UGANDA CANCER INSTITUTE

BY

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DISSERTATION SUBMITTED TO THE DIRECTORATE OF RESEARCH & GRADUATE TRAINING IN PARTIAL FULFILMENT OF THE AWARD OF A DEGREE OF MASTER OF SCIENCE IN IMMUNOLOGY AND CLINICAL MICROBIOLOGY OF MAKERERE UNIVERSITY

NOVEMBER, 2017

DECLARATION

I **Ocung Guido**, hereby declare that this research dissertation is the result of my own original work and that it has not been submitted in candidature for a degree or any other award in any university or institution.

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DEDICATION

This work is dedicated to my beloved mother Mrs. Margareth Anyinge Enangu, mentors: the late Emmanuel Ejumu and Paul Eweru (R.I.P), Emalu Peter, brothers and sisters for their continued support.

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LIST OF ABBREVIATIONS AND ACRONYM

AFI	-	Acute Febrile Illness
CRF	-	Case Report Form
DNA	-	Deoxyribonucleic Acid
EDTA	-	Ethylene Diamine Tetra Acetic Acid
ELISA	-	Enzyme Linked Immunosorbent Assay
HCMV	-	Human Cytomegalovirus
IgG	-	Immunoglobulin G
IgM	-	Immunoglobulin M
MBN	-	MBN Clinical Laboratories
PCR	-	Polymerase Chain Reaction
SST	-	Serum Separator Tube
UCI	-	Uganda Cancer Institute
UL	-	Unique Long Region of the viral genome

DEFINITION OF OPERATIONAL TERMS

Acute febrile illnesses (AFI): is defined as non-specific illnesses presenting with fever greater than 38°C lasting for less than two weeks without a readily diagnosable source after routine clinical evaluation

HCMV Infection: defined as prior exposure (based on a Positive IgG), recent infection (based on a positive IgM), and current active infection (based on a Positive IgM and DNA PCR).

Hematologic malignancies: are forms of cancer that begin in the cells of blood-forming tissue, such as the bone marrow, or in the cells of the immune system. Hematological malignancy will be used interchangeably with hematological cancer.

Persistent fever: is an episode of fever during neutropenia that does not resolve after 5 days of broad-spectrum antibacterial agents.

Seroprevalence: based on either IgG, or IgM, or both being Positive.

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ABSTRACT

Background: Sub-Saharan Africa is experiencing a marked increase in the burden of cancer-related morbidity and mortality, with more than 1 million incident cancers and nearly 800 000 cancer-related deaths projected annually by 2030. Among the patients with hematological cancers, chemotherapy as well as disease-specific factors are associated with the impairment of granulocyte number and function, predisposing patients to high risk of opportunistic infectious complications, which often manifest as fever. Among the viral infectious complications, Human Cytomegalovirus (HCMV) has been reported elsewhere to be a major opportunistic complication among patients with hematological cancers. However, limited data exists on the seroprevalence and contribution of HCMV infection among febrile patients with haematological cancers at the Uganda Cancer Institute (UCI).

Objective: To investigate the seroprevalence of HCMV exposure and active infection as well as risk factors for HCMV infection among hematological cancer patients with fever at the UCI.

Methods: In a cross-sectional study conducted between June and August 2017, blood samples were collected from 161 feverish patients receiving chemotherapy for various hematological cancers at the Uganda Cancer Institute. Detection of HCMV IgG and IgM as markers of infection was performed with an Indirect ELISA while a qualitative PCR was used to detect HCMV DNA extracted from whole blood at MBN Clinical Laboratories.

Results: Overall, HCMV seroprevalence based on IgG and/or IgM antibody positivity was found to be 106/161(66%). HCMV seroprevalence based on IgG or IgM antibody positivity was 84/161(52%) and 49/161 (30%), respectively. HCMV seroprevalence based on IgG alone, IgM alone, and combined IgG/IgM antibody positivity was 57/161(35.4%), 22/161 (13.6%) and 27/161(16.7%), respectively. HCMV DNA PCR positivity was detected in 5/161 (3%) of the samples. One of these was IgG alone positive, the other two were IgM alone positive while the remaining two where both IgG and IgM seropositive.

Conclusion: Overall seroprevalence of 66% was detected indicating that two thirds of the febrile patients with hematological cancers had been infected with HCMV, where active infection based on positive IgM and HCMV DNA PCR was detected in 23/161(14.3%) of the analysed samples. This result provides useful information to clinicians for proper management of patients with febrile illness on chemotherapy for underlying hematological cancers.

CHAPTER ONE: INTRODUCTION

1.1 Background

Cancer is considered the leading cause of death and disability worldwide and will soon eclipse infectious diseases within the next several decades if current trends continue. Based on GLOBOCAN estimates, there are 32.6 million people living with cancer (within 5 years of diagnosis), 14.1 million new cancer cases, and about 8.8 million deaths recorded worldwide as of 2015[1-3]. This burden has shifted to the less developed countries where it accounts for about 57% of the new cancer cases, 65% of cancer deaths and 48% of the 5-year prevalent cancer cases reported during 2012 [4]. Sub-Saharan Africa, in particular, is experiencing a marked increase in this burden, with more than 1 million incident cancers and nearly 800 000 cancer-related death projected annually by 2030, representing 85% increase from 2008[5]. However of these cancers occurring in sub-Saharan Africa, hematological malignancies have emerged as a major cause of morbidity and mortality that accounts for almost 10% of cancer death in the region [2, 5]. The progressive use of chemotherapy over many decades as well as disease specific factors are associated with the impairment of granulocyte number and function, predisposing such patients to viral, bacterial, fungal and parasitic infectious complication which often manifests as fever[5-7].

Fever is often the first and only sign of infection prompting the initiation of empirical antibacterial therapy [8-10]. Additional empirical antifungal therapy is often started in cases of persistent fever [11-13]. However, persistent fever lasting 4-5 days remains unexplained in 30 - 50% of febrile patients with no detectable evidence of clinically or microbiologically defined bacterial/fungal infection. Despite adequate antimicrobial/antifungal therapy hence suggesting that the fever is not necessarily related to the latter two infections but to viraemia as well [14, 15]. With concurrent presence of immunosuppression in patients with hematological malignancies, greater susceptibility to viral pathogens have emerged, which may result from the reactivation of latent infection or, rarely, from acquisition of a new infection [10, 16].

In the absence of effective antiviral prophylaxis, the incidence of Human Cytomegalovirus (HCMV) among patients with hematological malignancy ranges from 5-75%[17]. T-cell function is paramount in the control of HCMV, increased use of aggressive chemotherapy and T-cell depleting agents such as alemtuzumab used to treat cancer appears to increase the risk of HCMV disease in patients with hematological malignancy following its reactivation[16]. Initial infection is asymptomatic in the immunocompetent individuals; this is followed by latent state without active viral replication. However in immunocompromised subjects, primary infection is followed by a much more serious disease especially on those undergoing chemotherapy, where it manifest as febrile and sometimes life threatening disseminated disease[18, 19]. The risk for HCMV reactivation and severity of the resulting clinical manifestation ranges between 70-80% in patients with underlying hematological malignancy while those receiving outpatient regimen for solid tumors have a recurrent rate that is generally less than 10-50%[10, 20]. The impact of viral infection as a causative agent for fever in a setting with patients having underlying hematological malignancy has been less studied in Uganda.

1.2 Problem statement

Persistent fever not attributed to bacterial or fungal infections remains a problem among patients with underlying hematological malignancy. However, the contribution of the Human Cytomegalovirus (HCMV) as a cause of such fever remains poorly understood among patients at the Uganda Cancer Institute. Studies done in Brazil, from 2008-2010 assessed for HCMV seroprevalence in 470 patients with hematologic disorder patients, reported an overall HCMV seroprevalence of 89%. However, the study was limited to sickle-cell anemia, hemophilia, hemoglobinopathies [21]. Another study done among 68 children with acute lymphoblastic leukemia in Egypt (2001-2003) using ELISA, found a seroprevalence of HCMV IgG antibody 100% of either leukemic children or their control. However, this study was limited to children with acute lymphoblastic leukemia, it had a small sample analyzed and was done over 16years ago, and only assessed for HCMV prior exposure[22]. A recent study done in Sudan, 2015 that aimed at determining the seroprevalence of HCMV among 70 leukemic patient using ELISA, reported a HCMV IgG seroprevalence of 75%. However this study only focused on leukemic patient HCMV prior exposure status[23]. Since the above studies were done elsewhere, thus

cannot be used to inform the Ugandan situation due to the lack of data on HCMV. We investigated the burden of HCMV as a potential viral contributor to febrile illness in patients with hematological malignancies at the Uganda Cancer Institute.

1.3 Objective

I. To estimate IgG seroprevalence of HCMV exposure among febrile patients with hematological malignancy

II. To estimate the prevalence HCMV active infection based on a positive HCMV IgM and / or HCMV DNA PCR among febrile patients with hematological malignancy

III. To determine factors associated with HCMV active infection among febrile patients with hematological malignancy.

1.4 Justification

In the absence of appropriate methods to detect fever etiology, febrile patients continue to be empirically treated as having bacterial septicemia, malaria and fungal infection. Thus, the results obtained from this study provide useful information to clinicians for proper management of patients with febrile illness receiving chemotherapy for underlying hematological malignancies.

1.5 Research Questions

- What is the seroprevalence of HCMV exposure among febrile patients with hematological malignancy?
- What is the prevalence of HCMV active infection among febrile patients with hematological malignancy?
- Are there any factors associated with HCMV active infection among febrile patients with hematological malignancy?

CHAPTER TWO: LITERATURE REVIEW

2.1 Hematological malignancy

Hematologic malignancies are forms of cancer that begin in the cells of blood-forming tissue, such as the bone marrow, or in the cells of the immune system. Examples of hematologic cancers include the lymphomas (Hodgkin lymphoma and Non Hodgkin lymphoma), leukemia's (acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, and chronic lymphoblastic leukemia) and myeloma (multiple myeloma)[24, 25].

2.2 Persistent fever and febrile illness

Persistent fever is an episode of fever during neutropenia that does not resolve after five days of broad-spectrum antibacterial agents. The median time to defervescence following the initiation of empirical antibiotics in patients with hematologic malignancies is five days, in contrast with only two days for patients with solid tumors[26].

Acute febrile illnesses (AFI), are defined as non-specific illnesses presenting with fever greater than 38°C lasting for less than two weeks without a readily diagnosable source after routine clinical evaluation[27]

2.3 Viral infections among haematological malignancy patients

Viral infections are an important cause of morbidity and mortality of patients with haematological malignancy[16]. Over 130 herpesviruses of the *Herpesviridae* family are known and have been isolated from several animals. *Herpesvirales* order contains three families that include: *Herpesviridae*, *Alloherepesviridae* and *Malacoherpesviridae*. The *Herpesviridae* family also contains three subfamilies, *Alphaherpesvirinae*, *Betaherpesvirinae* and *Gammaherpesvirinae*[28]. Eight species of herpesviruses are known to infect human individuals, these include human herpesvirus 1 (HHV-1) or herpes simplex 1 (HSV-1), human herpesvirus 2 (HHV-2) or herpes simplex 2 (HSV-2), human herpesvirus 3 (HHV-3) or varicella-zoster virus (VZV), human herpesvirus 4 (HHV-4) or Epstein-Barr virus (EBV), human herpesvirus 5 (HHV-5) or cytomegalovirus (HCMV), human herpesvirus 6 (HHV-6A and HHV-6B), human

herpesvirus 7 (HHV-7) and human herpesvirus 8 or Kaposi sarcoma-associated herpesvirus (HHV-8 or KSHV) (see Fig. 1). Besides these, Macacine herpesvirus 1 can cause an infection in human individuals[29]

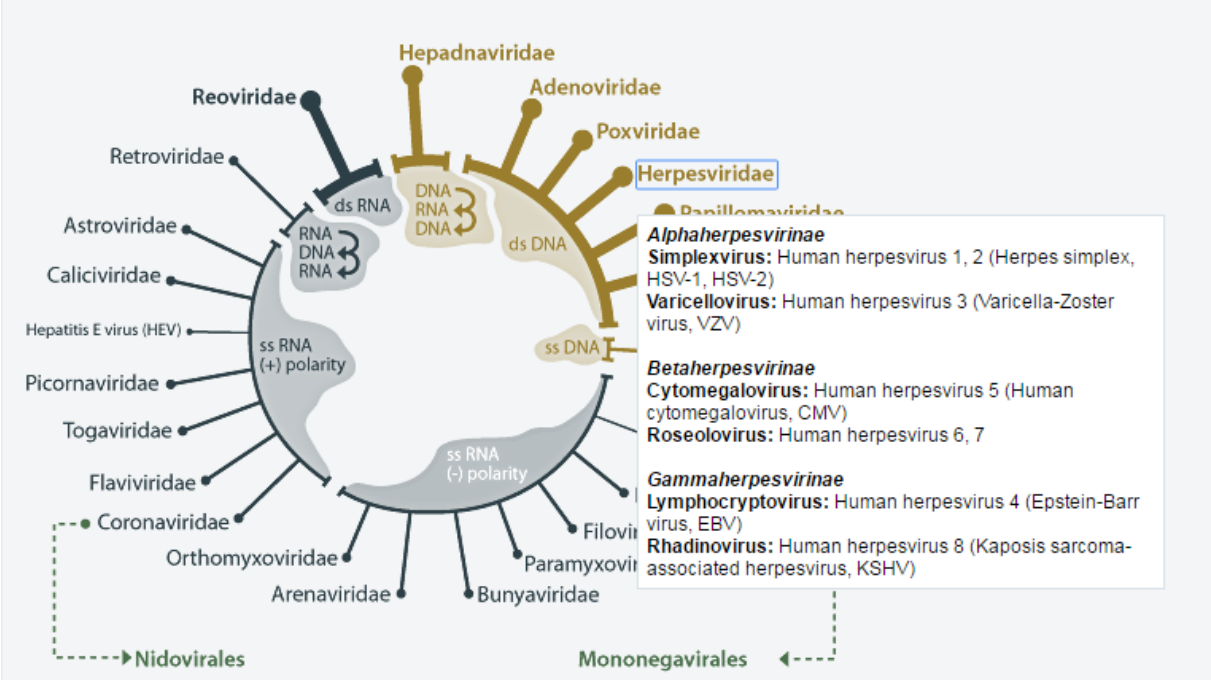

Figure 1: Taxonomy of herpesviruses.

(Source: https://www.antiviralintelistrat.com/1/Viral_Taxonomy)

The viral genome of these herpesviruses is double-stranded DNA (dsDNA) coated with icosahedral nucleocapsid. This nucleocapsid is covered by a pleomorph envelope. Between the envelope and nucleocapsid, tegument (matrix) can be observed (see Fig. 2.). On the basis of the microscopic morphology of herpesviruses, various species of herpesviruses could not be differentiated [29]. Members of *Alphaherpesvirina*e including HSV-1, HSV-2, and VZV have a short life cycle and these viruses can spread rapidly and cause mainly mucocutaneous infection, while for *Betaherpesvirinae* (HCMV, HHV-6 and HHV-7) the life cycle is slow; the infection is spread via saliva, genital secretes, blood or stem cell products, and these viruses may be latent in mononuclear cells. Gammaherpesvirinae including EBV and HHV-8 can establish latency in lymphoid cells and may cause a lytic infection in epithelial and fibroblast cells[29]. During infection, herpesviruses may enter the cells by endocytosis, the viral envelope fuses with the

membrane of endocytotic vesicle and the viral nucleocapsid is finally transported from the cytoplasm to the nucleus of the host cell. In the nucleus, the linear viral DNA will be circularised and DNA replication can begin. Viral gene expression is characteristic in herpesviruses and this process starts with the expression of immediate-early genes that code proteins regulating further gene expression. This is followed by the expression of early viral proteins that are necessary for DNA replication and protein phosphorylation; lastly late proteins are expressed, several of these being major structural proteins[29].

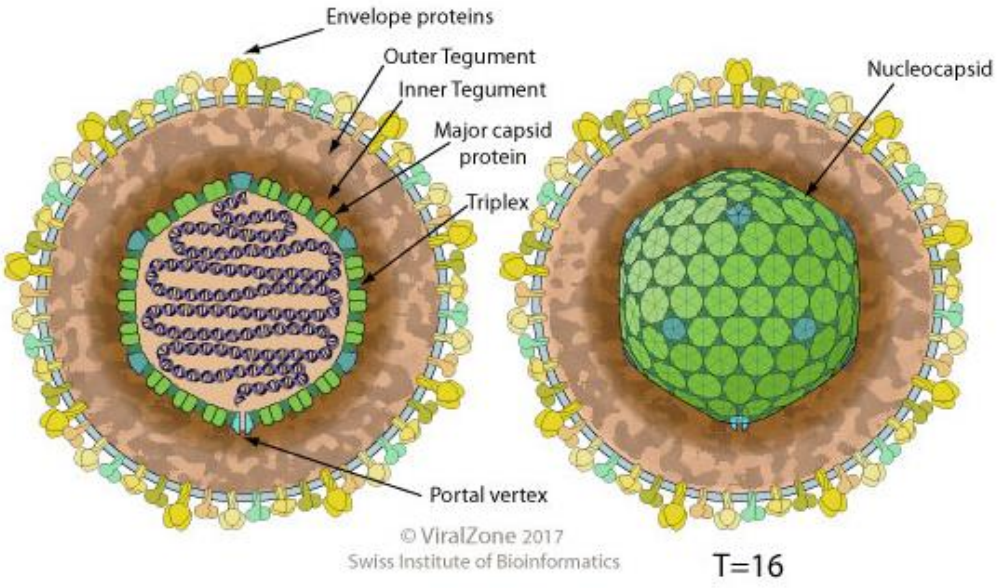

Figure 2: Structure of herpesviruses.

(source: http://viralzone.expasy.org/all_by_species/185.html)

2.4 The biology of Human Cytomegalovirus (HCMV)

Human Cytomegalovirus (HCMV), formally designated human herpesvirus 5 (HHV-5), is a member of the *Betaherpesvirinae* subfamily of the *Herpesviridae* family. This is the largest member of human herpesviruses that was first isolated from the salivary gland, while a description of the HCMV disease was first reported in 1965[29]. The structure of this virus is similar to that of HSV and VZV. The viral genome is linear dsDNA, which contains 164 non-overlapping open reading frames and it is completely sequenced. HCMV DNA is located in a

nucleoprotein core that is surrounded by a matrix protein and pp65 antigen. The HCMV genome has a single replication origin and contains a DNA polymerase gene (*UL54*). Another important gene is *UL97*, which encodes phosphotransferase enzyme. This enzyme can phosphorylate ganciclovir to ganciclovir monophosphate, this step being essential for ganciclovir to inhibit viral replication. HCMV also has several genes that can downregulate the host's immune response. HCMV enters the host cell via endocytosis, and like other herpesviruses the viral core is transported from the cytoplasm to the nucleus where after the synthesis of DNA polymerase, viral replication occurs. The consequence of viral replication is the development of nuclear viral inclusions[29]. HCMV infection as an endemic, seasonal variation could not be detected (i.e. it occurs all year round). After infection, the virus remains dormant in monocytes/macrophages. In humans, the virus can infect various cell types including epithelial-, endothelial-, neuronal-, smooth muscle-cells and fibroblast. Seropositivity rate changes lie between 40% and 90%, and they exceed 90% in the adult population. HCMV infection is often asymptomatic in a healthy individual, but sometimes a mononucleosis-like syndrome may occur in young adults. With cytomegalovirus mononucleosis, fever, lymphadenopathy and relative lymphocytosis like EBV mononucleosis may be observed, but the heterophile agglutinin test is negative, and a sore throat with enlarged tonsils is not characteristic; at the same time only low-level liver function abnormalities can be seen. In an immunocompromised host, HCMV-related disease may involve almost any organ, but the most common are the lung and gastrointestinal tract [16, 30, 31]. Interstitial pneumonia is one of the most frequent complications of HCMV infection in immunocompromised hosts - mainly in haematopoetic stem cell transplants -, and with this complication is associated a high mortality even with aggressive antiviral therapy. Another complication of HCMV infection mainly in immunocompromised patients is meningoencephalitis with motor and sensory weakness. In immunocompromised patients, the gastrointestinal manifestation of HCMV infection may be present as ulcers in the oesophagus and colitis with severe explosive watery diarrhoea. However, the incidence of HCMV infection and disease is less clearly defined among patients with malignant haematological disorders[32].

2.5 Laboratory diagnostic method for Human Cytomegalovirus

The laboratory diagnosis of HCMV infection is based on the culture of the virus taken from various body fluids or the detection of viral antigen or viral DNA or HCMV serological detection of specific antibodies. However, for our study, the description here will be limited to serological and DNA PCR methods.

2.5.1 Serology

Serologic assays are most useful for the identification of past infection; a positive assay for HCMV specific IgG indicates previous infection. Conversion from seronegative status to IgM positive is indicative of recent infection, and suggests that an acute illness may be associated with HCMV. However, when both HCMV IgM and IgG are positive, primary infection from reactivation cannot be definitively determined unless the patient's previous HCMV status was known. Many different assays have been described and evaluated for the detection of HCMV IgG antibodies. Among these are complement fixation, enzyme-linked immunosorbent assay (ELISA), anticomplement immunofluorescence, radioimmunoassay, and indirect hemagglutination [33]. Many different assays are available but enzyme-linked immunosorbent assays (ELISAs) are the most widely used and are based on crude viral preparations. The IgM capture assays are widely employed and are based on selective binding of IgM antibody to the solid phase. Recombinant IgM assays using recombinant HCMV proteins and peptides have been developed in an attempt to standardize serological assays[33]. Assays for IgM antibody lack specificity for primary infection because of false-positive results, as IgM can persist for months after primary infection, and thus remain positive in reactivated HCMV infections [34]. Due to the limitations of the IgM assays, IgG avidity assays are utilized in immunocompromised populations to help distinguish primary from non-primary HCMV infection[33].

2.5.2 Polymerase Chain Reaction Amplification

Molecular methods like real-time quantitative PCR have a higher sensitivity; therefore these techniques are more reliable diagnostic tools in immunocompromised patients[33]. With the use of molecular methods, it is possible to commence antiviral treatment in order to prevent the development of HCMV end-organ disease, so the use of the molecular method provides the basis

of pre-emptive therapy[33]. Specimen deterioration with time after sample collection is not as problematic with PCR assays as other tests for HCMV [33]. PCR for HCMV DNA can be either qualitative or quantitative. The threshold of the qualitative method needs to be carefully calibrated to prevent over-detection. Quantitative Real-Time PCR allows for continuous monitoring of immunocompromised individuals, to identify patients at risk for HCMV disease for preemptive therapy and to monitor their response to treatment[35, 36]**.** PCR is generally more expensive than antigenemia assays, but it is rapid and can be automated. Results are usually qualitatively reported as HCMV DNA Detected/Not detected or quantitate as number of copies/ml of blood or plasma[33].

2.6 Treatment and prevention of Human cytomegalovirus

Three major therapeutic strategies are used for managing HCMV infection, namely prophylaxis, pre-emptive therapy, and treatment of established disease[37]. Antiviral prophylaxis (the routine administration of antiviral drug for a fixed period at the patient's risk to prevent HCMV reactivation), pre-emptive therapy (based on the detection of viral reactivation by the molecular method, a pp65 antigenemia assay or culture; therefore the early introduction of antiviral therapy could prevent a progression to the HCMV disease), and treatment of established HCMV disease (based on the use of ganciclovir or foscarnet with the addition of immune globulin; cidofovir or the combination of ganciclovir and foscarnet can be use as second-line therapy) [16, 38]

Three antiviral drugs [ganciclovir (GCV), foscarnet, cidofovir (CDV)] have been shown to be efficacious and have been approved in the treatment of HCMV infection. The mechanism of action of these drugs involves the inhibition of viral DNA polymerase[16].

Ganciclovir is a guanosine nucleoside analogue, and this was the first effective antiviral drug against HCMV disease in human individual[39]. The UL97 gene of HCMV produces phosphotransferase, which converts GCV to GCV monophosphate, and it then is phosphorylated to GCV triphosphate. The triphosphorylated form of ganciclovir specifically inhibits the viral DNA polymerase. HCMV resistance to GCV is developed by point mutation of the UL97 gene, and the another type of GCV resistance is a consequence of mutation in the viral DNA polymerase gene [28]

Valganciclovir (VGC) is a prodrug of the GCV with a much higher bioavailability. Its oral form is equivalent to intravenous GCV. Foscarnet is a pyrophosphate analogue, and acts by direct binding to the viral DNA polymerase (CMV and other herpesviruses). Foscarnet is a treatment option for GCV-resistant CMV infection. It is administered intravenously, and it has a metabolic and nephrotoxic adverse reaction, such as renal failure, hypocalcaemia, hypomagnesaemia, hypophosphataemia[40], thus the close monitoring of serum creatinin and above-mentioned electrolytes levels, and supplementation of it are essential during therapy. Foscarnet resistant strains of CMV have been published because of the viral DNA polymerase gene mutation [40, 41]. Cidofovir (CDV) is a nucleotide analogue of cytosine with potent anti-CMV activity. The phosphorylation step is not necessary using a viral enzyme. CDV was found to be effective in the treatment of HCMV infection in patients undergoing allogeneic stem cell transplantation. CDV is also effective as a second-line therapy in relapsing cases after GCV or foscarnet treatment[28]

CHAPTER THREE: METHOD

3.1 Study design

This was a descriptive cross sectional study carried out between June and August 2017.

3.2 Study site and setting

The study was conducted at Uganda Cancer Institute (UCI). The UCI is the main cancer care and training centre in East African region currently serving Uganda, Kenya, South Sudan, Democratic Republic of Congo, Rwanda, and Burundi. UCI has a level six cancer ward with a capacity of 80 beds and attends to an average of about 200 patients daily. Patient recruitment was done at UCI. Immunological assays and molecular amplification laboratory studies on collected blood samples were done at MBN Clinical Laboratories using well qualified, competent and experienced personnel in molecular and life science analysis that were needed for this research work. The laboratory is a centre of excellence in infectious disease diagnosis with a core function of offering medical laboratory diagnostic services. This facility applies molecular techniques, culture and drug sensitivity, immunological and histopathological tests to diagnose disease.

3.3 Study population

Patients with hematological malignancy on chemotherapy who presented with febrile illness at Uganda Cancer Institute between the months of June 2017 to August 2017

Eligibility: The study was open to both children and adults of either gender.

Inclusion criteria

Adult and pediatric patients with confirmed diagnosis of hematological malignancies who had been on cancer chemotherapy for at least four weeks

An axillary (under the arms) temperature greater than 37.5°C

Provision of a written informed consent by volunteers and assent from parents/guardians of children older than 8 year

Exclusion criteria

Patients who were unconscious and unable to provide a written informed consent were excluded from this study.

3.4 Sample size

A non-random convenient sampling frame was used in this study; from the day the study started, eligible volunteers with acute febrile illness on chemotherapy from both the Out-Patient Department and Cancer Ward of Uganda Cancer Institute (UCI) upon providing an informed consent were consecutively enrolled into study until an appropriate sample size of 161 was attained.

3.4.1 Sample size calculation

A sample size of **161** was selected based on the formula obtained from Kish, Leslie (1965).

$n = (Z^2 PQ)/e^2$

Where n is the sample size required.

Z^2 – Is the area under the standard curve with a CI of 95%.

P – Is the proportion of the population with the disease [Previous Prevalence of HCMV infection in such a study population done in the sub-Saharan Africa was 90%][42]

Q – Is the proportion of the population without the disease (1-P)

e^2 – Is the square of the precision of the testing kit used.

Using the prevalence of 90%, CI of 95%, e of 0.95, then Q = (1 – 0.25)

$n = [(1.96)^2 \times 0.90 \times 0.75] \div (0.95)^2$

n = 288.

However since the clinic at Uganda Cancer Institute receives less than 200 patients with febrile illness per month, a total sample size of 288 would not be attained in a one month. Therefore, an overall sample size of **161** was used for this study.

3.5 Sampling Technique

Consecutive sampling technique was used in recruiting participants into the study because it was simpler and thus reduced the study period hence making it a much cheaper framework model to suite within the shorter reporting time narrowed by the school schedule. This study was thus bound to selection bias due to the sampling technique used.

3.6 Methodological details

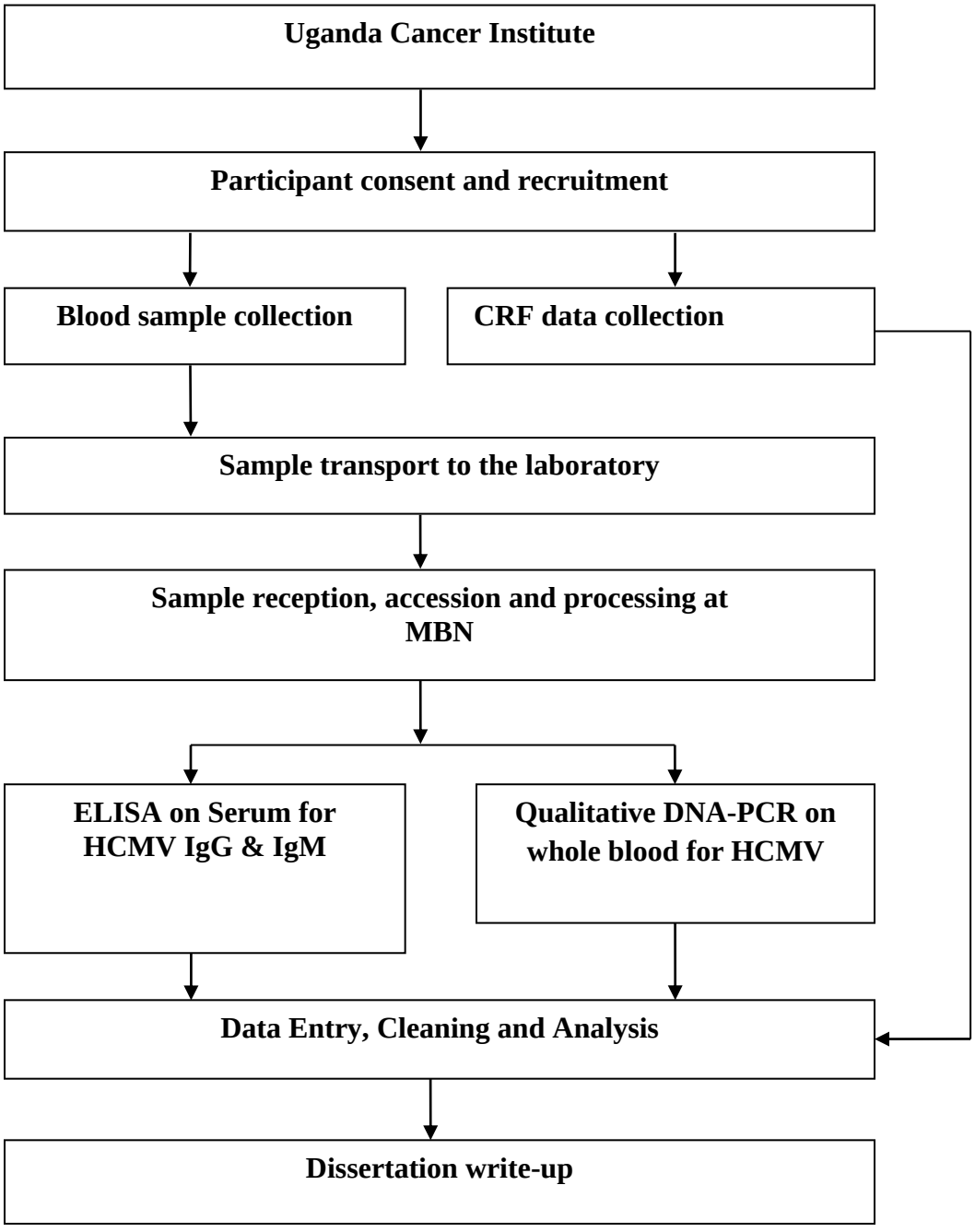

Figure 3: Flow chart summarizing the nature of work that was done in the course of the study.

Where CRF = Case Report Form. ELISA= Enzyme Linked Immunosorbent Assay.

IgG = Immunoglobulin G. IgM= Immunoglobulin M. HCMV= Human Cytomegalovirus

3.6.1 Consent and patient recruitment

Patients were approached and the study was explained to them so as to seek for their consent to voluntarily participate in the study. Children whose parents or care takers (guardians) voluntarily accepted to have them take part in this study were requested to provide a written informed consent and assent from children aged 8 years and above before recruitment. Participant recruitment was conducted from Monday to Sunday between 8am to 5pm at UCI.

3.6.2 Data collection tool

A standardized Case Report Form (CRF) was used to collect participants' information from the available medical file. This tool did capture information in the key areas of socio-demographics, type of hematological malignancy and chemotherapy regime received as elaborated further in the attached appendix IX

3.6.3 Sample collection and storage

Venous blood was drawn aseptically from the participants within 72 hours of fever onset. Where 2ml from adults and 2ml from children of the blood sample were collected into Serum Separator Tube (SST) vacutainer, followed by Ethylene Diamine Tetra-acetic Acid (EDTA) vacutainer tube (in total, 4ml of blood draw were obtained from both adults and children). Collected samples in both EDTA and SST tubes were labeled in the presence of the study participant with their unique study ID and stored at ambient temperature of between 24-26°C prior to delivery to the laboratory for further processing.

3.6.4 Sample transport

Blood samples in the primary vacutainer tubes were individually packaged in a leak-proof zip-lock bag with sufficient absorbent material to absorb any content should leakage occur. These were transferred into a cool box whose interior and external surface had been disinfected with 70% alcohol. A filled chain of custody sample transfer documentation in a sealed envelope was attached to the external surface of the cool box using masking tape then delivered to the processing laboratory within one hour from collection time.

3.6.5 Sample reception, accession and processing at MBN clinical laboratory

Specimens collected from UCI were logged (accessioned) onto the Laboratory Information Management System, where individual specimens again were subjected to thorough verification to the corresponding details on specimen processing requisition form (Chain of Custody) for missing information, any leakage, and transportation temperature condition. Blood samples in SST were centrifuged at 400xg for 10 minutes to separate out serum. The serum was then aliquoted into a cryogenic sample tube appropriately labeled with the unique laboratory Identification number, specimen type, study number and storage date then stored at -20°C pending testing. The samples in EDTA tube were temporarily stored at 4°C pending DNA extraction. Specimen Biorepository is located at the MBN Clinical Laboratory that did maintain a cumulative inventory list (Number of vials/specimen) for all stored study specimen. The Laboratory Information Management system in place was used to assign storage space, position for frozen samples as this will facilitate efficient retrieval of archived specimen for use in future studies.

3.6.6 Laboratory Methods

The laboratory tests in this study included ELISA for HCMV IgG and IgM, and Qualitative PCR for HCMV DNA.

3.6.6.1 Enzyme Linked Immunosorbent Assay for both HCMV IgG and IgM

Anti-HCMV IgG and IgM were individually measured using an Indirect-ELISA kit (Diagnostic Automation, Inc (21250 Califa Street, California 91367 USA) according to the manufacturer's instructions. Purified HCMV antigens were coated on the surface of microwells. Diluted patient serum was added to wells, and the HCMV IgG or IgM specific antibody, if present, binds to the antigen. All unbound materials were washed away. After adding enzyme conjugate, it binds to the antibody-antigen complex. Excess enzyme conjugate was washed off and TMB Chromogenic substrate added. The enzyme conjugate catalytic reaction was stopped at a specific time. The intensity of the color generated was proportional to the amount of HCMV IgG or IgM specific antibody in the sample. The results were then read by a microwell reader and compared in a parallel manner with the incorporated calibrator and controls.

An ELISA index of 1.0 or greater was considered positive. Samples were considered negative if ELISA index was less than 0.90. Results were considered equivocal if the ELISA index was between 0.91and 0.99.

3.6.6.2 Qualitative PCR for HCMV DNA

This involved extraction of genomic DNA from whole blood, PCR reagent mix preparation, amplification and detection of HCMV DNA on gel electrophoresis.

Extraction of DNA from whole blood

DNA was extracted from 150µl of whole blood lysed in 100µl of 10% sodium dodecyl sulfate and then incubated at 65°C for 10 minutes, followed by 100µl of 3N Sodium acetate. The supernatant was subsequently purified by phenol-chloroform extraction and ethanol precipitation. The dried pellet containing the DNA was then eluted in 100µl of PCR water and stored at -20°C until use.

PCR Reagent Mix

In the Pre-PCR laboratory, HCMV PCR master mix reactions were set up as indicated in Table 1

Table 1: HCMV PCR master mix

PCR recipe	PCR Reaction vol (µl)	PCR Reaction vol (µl) for N samples
PCR water (promega)	7.0	7.0N
PCR custom mix (10X),(thermofisher)	1.5	1.5N
Q-solution (Qiagen)	1.5	1.5N
Magenesium chloride (25mM) (Qiagen)	0.8	0.8N
CMV US8 F (100ng/µl), Soeten et al,2008	1.5	1.5N
CMV US8 R (100ng/µl), Soeten et al,2008	1.5	1.5N
Taq polymerase (5units/µl), (Qiagen)	0.2	0.2N
Total	14.0	14.0N

For the detection of HCMV DNA, PCR primers targeting the non-coding US8 region as previously described by Soeten et al,[43] were obtained from integrated DNA technologies (Coralville San Diego, CA USA). The PCR primers sequences used in this study are indicated in the Table 2.

Table 2: Primer sequence used in the detection of HCMV DNA

Primer Name	Sequence (5' – 3')
CMV US8 F	GGATCCGCATGGCATTCACGTATGT
CMV US8 R	GAATTCAGTGGATAACCTGCGGCGA

Amplification

To 14.0µL of the PCR reaction mix was added 10.0µL of the eluted DNA and the sealed PCR tubes transferred to Amplification laboratory where a GTQ 96 thermocycler (Hain Life Sciences) was used to amplify the DNA template. The conditions were set to 35 cycles of DNA denaturation at 95°C for 30 seconds, annealing at 55°C for 20 minutes, and extension at 72°C for 1 minute 30 seconds as summarized in Table 3.

Table 3: Cycling profile for HCMV DNA amplification

PCR steps	Temperature (^{0}C)	Time (secs)	Cycles
1 (Initial Denaturation)	95	300	1
2 (Denaturation)	95	30	35
3 (Primer Annealing)	55	20	
4 (Extension)	72	90	
5 (Store)	4.0	∞	1

Detection on Gel Electrophoresis

The reaction product was resolved by electrophoresis using 2% agarose gel (Sigma) in 1% Sodium Borate buffer stained with 7.5µl of 5mg/ml ethidium bromide, run at 120 volts (constant voltages, variable current) and examined under UV transilluminator and photographed. Positive (Plasmid) and negative (PCR water) controls were included for every experimental run

performed. The band at the 409bp fragment was considered Positive for HCMV DNA as shown in Figure 4.

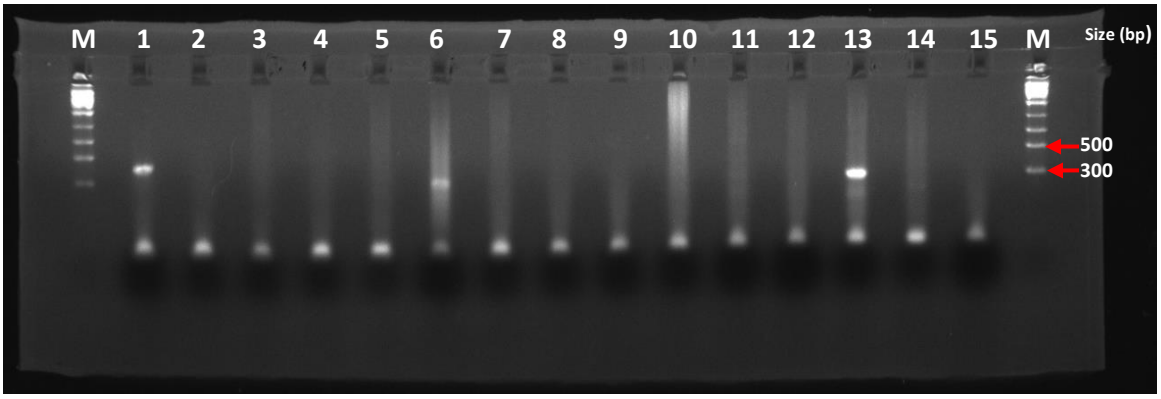

Figure 4: Agarose gel electrophoresis results.

Lanes: M=1Kb ladder (Solis Biodyne), 1=Positive control, 2=Negative control (PCR water); lanes: 3, 4,5,7,8,9,10,11,12,14 and 15 were Negative for HCMV, lanes: 6 and 13 were positive for HCMV.

3.6.7 Study variables

The following independent variables were assessed.

Socio-demographic characteristics of study participants includes: Sex, age, level of education, type accommodation, and number of occupants, geographical location (where the participant stays/there current address

Clinical characteristics of the study participants includes: Type of hematological malignancy and immunosuppressive treatment use, HIV serostatus, clinical history of other bacterial and parasitic infection especially with malaria

Interventions received by the study participants include: Chemotherapy regimen, number of phases/cycles of the chemotherapy regime received, antimalarial and /antibiotic prophylaxis use in the last 72 hours, and blood transfusion history.

3.6.8 Study Outcome

The following dependent variables were assessed.

1. Proportion of Positive HCMV IgG among febrile patients with hematological malignancy.
2. Proportion of Positive HCMV IgM and DNA PCR among febrile patients with hematological malignancy.

3.7 Data management

3.7.1 Data entry

Patient demographic data was collected by research assistants using a pretested standardized case report forms (CRFs). All CRFs and laboratory reports were reviewed for purposes of ensuring both the correctness and completeness before data entry. Changes and corrections to the CRFs were neatly crossed using a single line then initialed and dated following the Good Clinical Practice (GCP) guidelines. Data on other participant's demographics captured on a standardized CRF and the data generated from the laboratory reports were subjected to double data entry into Epi Data version 3.1. To minimize data entry errors, a data entry template was used to restrict the range of values that could be entered for any data item with mandatory entry for all data fields.

3.7.2 Data cleaning

All data were subjected to double entry for review of data entry error level. Each CRF and generated laboratory reports were assigned a unique identifier to allow for validation. Source documentation was available for review to ensure that data collected and recorded from the laboratory database and CRFs were consistent with the contents of the source documents.

3.7.3 Data storage

To ensure the confidentiality of the study data, access to the data was restricted by use of passwords only available to the principal investigator and other persons nominated by him. When not in use, paper copies of the results and data collection forms were kept in a locked filing cabinet in a secure room. The computer record files were saved regularly, especially, during data entry to prevent loss of data due to technical difficulties. All computer files were backed-up, and paper copies printed, at least once a day during data entry.

3.7.4 Quality control

In order to ensure the quality and reliability of the data gathered and ethical conduct of this study, the following measures were undertaken; Standard Operating Procedures (SOP) in place were adopted for all laboratory analytical procedures. Data collection tools were pre-tested prior to commencement of the study to ascertain that the required information could be obtained with ease. Accuracy of data collected was ensured by thorough cross checking of the entered soft copy version against source data. Regular monitoring and monthly reporting of the study progress to the Chair, Department of Medical Microbiology at Makerere University College of Health Sciences.

3.7.5 Data analysis

Demographic characteristics, clinical and laboratory data were coded and entered into Epi Data version 3.1 data capture tools followed exportation to STATA version 14(Stata Corp LP, College station, Texas) for analysis and then presented in the form of descriptive statistics. Baseline demographic characteristics of the volunteers; continuous variables were summarized as mean and median for data that was not normally distributed whereas categorical variables were presented in form of proportions.

To answer objective 1 and 2: Estimate IgG seroprevalence of HCMV and determine its active infection based on a positive HCMV IgM and/ HCMV DNA PCR among febrile patients with hematological malignancy. The results of this study collected from laboratory test were analyzed as follows: number and proportion of patients with positive test results for HCMV were generated and prevalence of both HCMV prior and active infection among patients with febrile hematological malignancy summarized in form of percentage after cross tabulation and presented in Table 5, and Figure 5.

To answer objective 3: Determine factors associated with HCMV active infection among febrile patients with hematological malignancy. Clinical and routinely collected laboratory data were extracted from all consented participant's medical records using a standardized Case Report Form (CRF). Bivariate analysis was performed using Fischer's exact/ Chi square test for categorical data where factors with p-value <0.2 in Table 6, were considered for multivariate

analysis. Forward conditional binary logistic regression analysis was performed with the presence of HCMV IgM as an independent variable for all parameters described in Table 7. All predictor values with a *P-value* <0.05 were considered as statistically significant.

3.8 Ethical consideration

Approval and clearance to carry out this research was sought and obtained from the School of Biomedical Sciences Higher Degrees Research and Ethics Committee (SBS-HDREC), Makerere University College of Health Science, Kampala. Administrative clearance for the conduct of this study was obtained from UCI. Written informed consent was sought from each volunteer before being enrolled into the study. Laboratory results were availed to the attending doctors for appropriate management of the patients. Patient records are to be kept under lock and key for the next three years and only availed to authorized persons following the Good Clinical Practice (GCP) guidelines. Information obtained was treated with utmost confidentiality. Patient information has been detached from one to be published.

3.9 Result dissemination plan

The results of this study were disseminated to the Department of Microbiology, Directorate of Research and Graduate Training (DRGT) - Makerere University, Sir Albert Cook Medical School Library, Uganda Cancer Institute, Ministry of Health to guide policy making, published in a suitable peer-reviewed journals, and presented at local and international conferences.

CHAPTER FOUR: RESULTS

4.1 Socio demographic and clinical characteristics of the participants:

A total of 161 participants with hematological malignancy presenting with febrile illness were evaluated for HCMV infection between June and August 2017.

Of these 86(53%) were females. The median age in the study was 29years [IQR 17-43]. Here, 128(80%) were on intensive chemotherapy regimen while 33(20%) had received the less intensive chemotherapy regimen. Table 4. Summarizes participants socio demographic and clinical characteristics.

The distributions of the studied underlying hematological malignancy were as follows: 43(27%) had Acute Lyphoblastic Leukemia, 30(19%) had NHL and AML Hodgkins Lymphoma and Chronic Myeloid Leukemia also had and 18(11%) patients had HL and CML. 12(7%) and 10(6%) enrolled participant had MM and CLL respectively as shown in Figure 5 below.

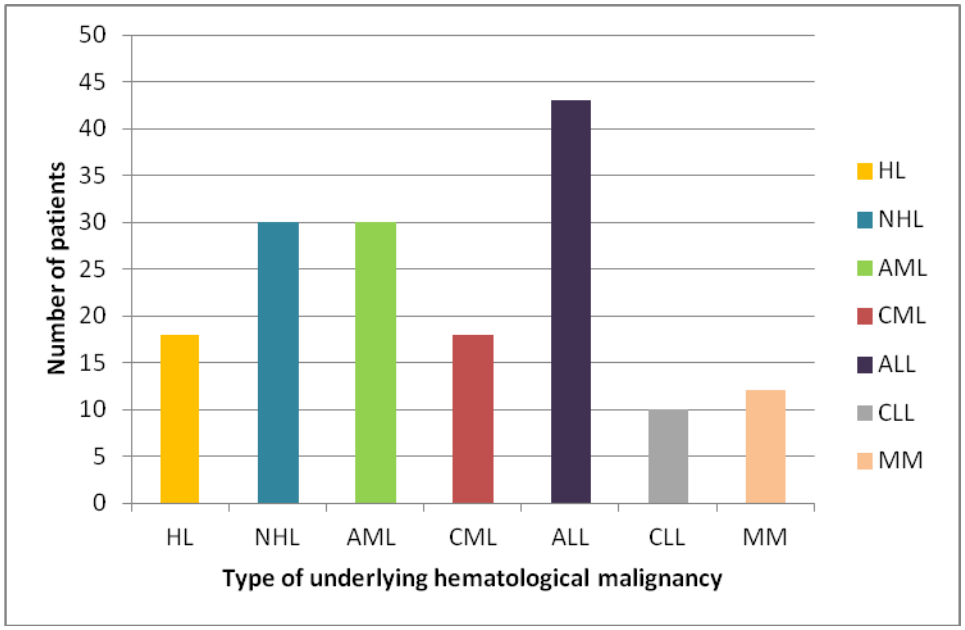

Figure 5: A bar graph depicting the distribution of the studied hematological malignancy.

Key:
ALL-Acute Lyphoblastic Leukemia
AML-Acute Myeloid Leukemia
CLL- Chronic Lyphocytic Leukemia
CML-Chronic Myeloid Leukemia
HL-Hodgkin Lymphoma
MM - Multiple Myeloma
NHL - Non Hodgkin Lymphoma

Table 4: Socio-demographic and clinical characteristics of the participants

N=161	n (%)
Gender: Male	75(47)
Female	86(53)
Age, years, Median[IQR]	29[17-43]
0 to 17 years	42(26)
Above 18 years	119(74)
Rx: Intensive	128(80)
Less intensive	33(20)
Hematological malignancy	
HL	18(11)
NHL	30(19)
AML	30(19)
CML	18(11)
ALL	43(27)
CLL	10(6)
MM	12(7)
Education: None	12(8)
Primary	55(34)
Secondary	52(32)
Tertiary	42(26)
Household occupants	
0 to 6	89(55)
7 and above	72(45)
Intervention	
Antibiotic	114(71)
Blood transfusion	113(70)
Steroid therapy	120(75)
HIV status: Unknown	13(8)
Positive	14(9)
Negative	134(83)

Key:

ALL-Acute Lyphoblastic Leukemia
AML-Acute Myeloid Leukemia
CLL- Chronic Lyphocytic Leukemia
CML-Chronic Myeloid Leukemia
HIV- Human Immunodeficiency Virus
HL-Hodgkin Lymphoma
IgG - Immunoglobulin G
IgM - Immunoglobulin M
IQR- Interquartile Range
MM - Multiple Myeloma
NHL - Non-Hodgkin Lymphoma
Rx- Chemotherapy regime

4.2 Seroprevalence of HCMV IgG and IgM

Of the 161 febrile patients evaluated with hematological malignancy, HCMV seroprevalence based on IgG and/or IgM antibody positivity was found to be 106/161(66%). HCMV seroprevalence based on IgG antibody positivity was 84/161(52%) and IgM positivity 49/161 (30%), respectively. HCMV seroprevalence based on IgG alone, IgM alone, and combined IgG/IgM antibody positivity was 57/161(35.4%), 22/161 (13.6%) and 27/161(16.7%), respectively as shown in Table 5 and Figure 6.

Table 5: Seroprevalence of HCMV

Serological Results	No. of seropositive (%)
HCMV-IgG and/ or IgM	106(66%)
HCMV-IgG	84(52%)
HCMV-IgM	49(30%)
HCMV-IgG alone	57(35.4%)
HCMV-IgM alone	22(13.6%)
Combined HCMV IgG and IgM.	27(16.7%)

N=**161**

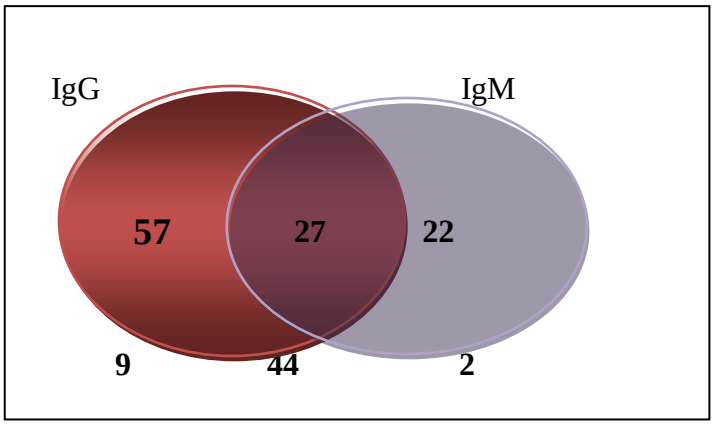

Figure 6: A Venn diagram depicting HCMV-IgG and IgM results.

HCMV DNA was detected in 5/161(3%). Of these, one had a positive IgG alone, the other two were positive for IgM alone while the remainining two where seropositive for both IgG and IgM.

4.3 Risk factor analysis for HCMV active infection.

In Bivariate analysis, seven variables had p-value <0.2 as indicated in Table 6.

Table 6: Bivariate analysis using Fisher Exact Chi-square test

N=161	Chi-square	P-value
Gender	1.3644	0.534
Age	2.1316	0.444
Education	8.7046	**0.141**
Household occupants	6.2612	**0.037**
Chemotherapy regime	1.1469	0.734
Hematological malignancy		
HL	4.8663	**0.062**
NHL	4.1296	**0.097**
AML	0.8530	0.481
CML	2.2117	0.421
ALL	2.3826	0.387
CLL	2.5627	0.363
MM	0.5692	0.821
Intervention		
Antibiotic	4.1742	**0.154**
Blood transfusion	0.2967	0.878
Steroid therapy	11.9211	**0.003**
HIV status	10.3114	**0.104**

Key:

ALL-Acute Lyphoblastic Leukemia
AML-Acute Myeloid Leukemia
CLL-Chronic Lyphocytic Leukemia
CML-Chronic Myeloid Leukemia
HIV-Human Immunodeficiency Virus
HL-Hodgkin Lymphoma
MM-Multiple Myeloma
NHL-Non Hodgkins Lymphoma

In multivariate analysis, only steroid therapy had a *P-value* that was less than 0.05 (OR 0.36, 95% CI 0.17-0.79, P = 0.01) as shown in Table 7.

Table 7: Multivariate logistic regression analysis of risk factors for active HCMV infection

N=161	**Adjusted OR**	***P-value***	**95% CI**
Education	0.83	0.35	0.56-1.23
Household occupants	1.82	0.11	0.87-3.81
Hematological malignancy			
HL	2.44	0.10	0.84-7.06
NHL	0.76	0.59	0.29-2.02
Intervention			
Antibiotic	1.17	0.71	0.51-2.67
Steroid therapy	0.36	**0.01**	0.17-0.79
HIV status	1.00	0.99	0.55-1.82

Key: *HL- Hodgkins Lymphoma, NHL- Non Hodgkins Lymphoma*

CHAPTER FIVE: DISCUSSION

5.1 Discussion

In this cross-sectional study, we investigated the burden of Human cytomegalovirus in patients with hematological cancers on chemotherapy presenting with febrile illness at Uganda Cancer Institute.

Where an overall HCMV seroprevalence based on IgG and/or IgM antibody positivity was found to be 106/161(66%). While HCMV seroprevalence based on a positive IgG alone was detected in 57/161(35.4%), which is suggestive of prior infection with HCMV. This finding is in concordance with earlier studies from Sudan by Dafalla., 2015 and de Matos et al .,2011 from Brazil who reported an HCMV IgG seroprevalence of 76% among Leukemic patients and 89% in hematologic disorder patients respectively[21, 23]. After a person has been exposed to HCMV, they will have some measurable level of HCMV IgG antibody in their blood for the rest of their life. And as such HCMV IgG antibody testing should be done, alongside HCMV IgM testing, to help confirm the presence of a recent or previous HCMV infection.

In our study, a positive HCMV IgM alone was observed in 22/161 (13.6%) of the analysed serum samples. The positive HCMV IgM result indicates a recent infection (primary, reactivation, or reinfection). This is consistent with other studies done in Nigeria and in Egypt by in which (26%) and (19%) HCMV IgM seropositivity were reported respectively among patients undergoing chemotherapy for hematological malignancy[42, 44]. HCMV IgM results alone should not be used to diagnose HCMV infection. In case of suspected active HCMV infection, this may need to be confirmed through molecular detection HCMV DNA.

In our study, HCMV DNA PCR positivity was detected in 5/161 (3%) of the analysed whole blood samples. Among the five, one was IgG alone positive; two were IgM alone positive while the remainining two were both IgG and IgM seropositive. A positive IgG and DNA PCR is suggestive of reactivated latent infection, such individuals are considered more susceptible to primary infection. Positive IgM and DNA PCR indicates recent infection (primary, reactivation, or re-infection). When both HCMV IgM and IgG are positive, this may imply a seroconversion to IgM positive which is indicative of recent infection, and thus suggests that the acute illness

may be associated with HCMV as confirmed by the positive DNA PCR. However, a primary infection from reactivation or re-infection cannot be definitively determined unless the patient's previous HCMV status was known.

These findings are consistent with those found in other earlier published observations by Daniel et al.,2016 in which 16/169(9.5%) and Sheen et al., 2009 in which 26/252(10.3%) samples were reported as being positive for HCMV DNA[45, 46]. HCMV IgG and IgM results should not be used alone to diagnose HCMV infection. However, such results need to be considered in conjunction with clinical presentation, patient history and other laboratory finding. In this context therefore, testing for HCMV using DNA-PCR in hematologic malignancy patients presenting with febrile illness may prove very a useful technique in the rapid diagnosis of active HCMV infection thus prompting pre-emptive antiviral therapy against HCMV. This therapeutic strategy unlike prophylaxis and treatment for established HCMV disease has the advantage for preventing progression to end-organ disease, reduces exposure to antiviral toxicity, and maximizes cost benefit ratio.

In multivariate analysis, only steroid therapy had a *P-value* of less than 0.05 (Adjusted OR 0.36, 95% CI 0.17-0.79, P = 0.01). Indicating that its protective against HCMV active infection among febrile patients with hematological malignancy.

Several inherent limitations to our cross-sectional study design deserve to be acknowledged. First capturing data at a single time point limits the ability to assess the temporality of virological burden in relation to HCMV disease. Second, although we found 30% HCMV IgM seropositivity during febrile events in 161 patients with underlying hematological malignancy, we do not have data during periods without fever and therefore cannot be certain that 3% HCMV viremia (based on the observed positive DNA PCR) which is specifically associated with febrile illness. Identification of possible causes of febrile illness was limited by the assays we performed and thus cannot exclude other viral pathogens for which no testing was conducted. Finally, although a limited number of a substantially heterogeneous patient population was studied, the lack of other identified agents coincident with fever suggests that HCMV may be the probable cause for persistent febrile illness lasting more than 4 days in this population despite adequate empiric antimicrobial therapy.

CHAPTER SIX: CONCLUSION AND RECOMMENDATION.

6.1 Conclusion

In conclusion the overall prevalence of 66% was detected indicating that two thirds of the febrile patients with hematological cancers had been exposed to HCMV, while current active infection based on positive IgM and HCMV DNA PCR was detected in 23/161(14.3%) of the analysed samples. This result provides useful information to clinicians for proper management of patients with febrile illness on chemotherapy for underlying hematological cancers.

6.2 Recommendation

From our study findings coupled with other observation in a similar setting, we recommend the inclusion of HCMV in the differential diagnosis for febrile illness in patients with underlying hematological malignancy especially those presenting with persistent fever despite adequate antimicrobial therapy.

Further studies, including the investigation of other pathogens need to be performed so as to understand better the scope and impact of viral reactivation in febrile patient undergoing chemotherapy for underlying hematological malignancy.

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APPENDIX I: INFORMED CONSENT FORM

Patient Informed Consent in English

1. Title of the proposed study:

Human Cytomegalovirus in febrile patients with underlying hematological malignancies at Uganda Cancer Institute

2. Investigator:

Ocung Guido, a Master student from Makerere University College of Health Sciences, Kampala

3. Introduction:

- You are being approached (requested) for your consent to take part in a research study
- This consent form gives you details about the research study. You can read it or it may be read to you and you are free to ask any question about anything that you may not understand.
- After you have understood and decided to take part in this research study, you will be required to sign this consent form. A copy of which will be given to you.
- Your participation in this research study is voluntary. Even if you decide not to participate you will still continue to receive care at the institute and will not be penalized.
- Upon signing the informed consent form, you will be asked a few questions, followed by body examination/checking for any illness before 2 table spoonsful of blood can be drawn from you for testing of cytomegalovirus (a virus that causes fever besides other germs)
- You have a right to withdraw your consent for study participation at any time and for no reason.

4. Background and rationale for the study:

The reason this research study is being carried out is that, patients with cancer of the blood tend to have persistently raised body temperature despite adequate antibacterial/antifungal treatment

medication. Therefore, by knowing what cytomegalovirus is there it can help guide the clinician to administer the most appropriate drugs

5. Purpose of the study:

This study is being conducted in both children and adults with cancer of the blood that show up with a raised body temperature with the aim to find out how many of them have cytomegalovirus in their blood.

6. Study procedures:

- If you agree to join the study, and after you have signed this consent form, your time in this study will last for only a few minutes.
- You will be asked a few questions concerning where you stay, your name, age, and others.
- You will then be examined and blood samples collected from you for the detection of cytomegalovirus germ using a safe method.
- A form used to record this information will be kept secret at all times.
- We would also like to know your HIV status so that you can be linked to a facility where you can access care in case you are found to be positive for HIV.

7. Who will participate in the study?

161 children and adult patients with cancer of the blood that show up with raised body temperature upon voluntary consent will be studied

8. Risks/Discomforts:

- You may get some discomfort when you are being asked some questions. However, you are free not to answer any question you may not be comfortable with.
- You may experience some mild pain during blood draw and discomfort like bruises. However, this will be minimized by ensuring that the study sample is taken off at the same time your doctor collects blood for your routine care.

9. Benefits:

You may not directly benefit from this study however the information obtained will help guide care providers in the management of patients that show up with persistently raised body temperature in the near future.

10. Alternatives:

You do not have to take part in this study if you do not want to. You will still continue to receive your medical care as before.

11. Cost:

There will be no costs to you as you take part in this research study.

12. Reimbursement:

You will not be paid for study participation. However, you will be given **10,000/=** Ugandan shillings as a contribution for your time spent while taking part in this study.

13. Contacts for concerns/Questions:

If you have more questions and information that you may need clarifications on, please contact the Principle Investigator: **Ocung Guido** Tel **+256 754 736312 Email: guidoocung@gmail.com**

In case you have concerns regarding your rights as a participant, please feel free to contact the **Chairperson, School of Biomedical Sciences Higher Degrees Research & Ethics Committee [SBS-HDREC], Dr. Erisa Mwaka** Tel **+256 752575050** Email:**erisamwaka@yahoo.com**

14. Statement of voluntariness:

Enrollment into this study is voluntary and has no penalty incurred for your participation. You are free to decline to take part in this research study or withdraw from the study at any time and this will not affect your management.

15. Confidentiality:

The results of this study will be kept strictly in secret, and used only for research purposes. Your identity will be kept secret and prevented from being known in as far as the law allows. A code will be used in place of your name for most of the study documents. Paper and computer records will be kept under lock and key and with password protection respectively. If the results of this research are published your names will not be shown.

16. What signing or putting a thumb print on the consent form means

You must understand that by signing this form, you do not waive any of your legal rights but merely indicate that you have been informed about the research study in which you have agreed to voluntarily take part in. Signing often simply means that you have understood information in the consent form and thus accept to participate in the research study.

17. Statement of Consent

I do acknowledge reading the information in this consent form or the information has been read and explained to me, I do understand the purpose of the study, what is going to be done, the risks, the benefits involved and my rights regarding study participation/consent withdraw, access to care, and confidentiality and I have voluntarily accepted to take part in this research study.

---------- ---------- ----------

Name of participant (Print) Signature/thumbprint Date

To those that use thumb prints for consent (Only):

I attest that the participant's name is----------and has placed his/her thumbprint on this consent form on this date----------

---------- ---------- ----------

Name of witness (Print) Signature Date

---------- ---------- ----------

Name of study staff obtaining the consent (Print) Signature Date

APPENDIX II: SAMPLE STORAGE CONSENT FORM.

Informed Consent Form for storage and future use of unused samples in English.

1. Purpose of sample storage

- You are being requested to allow your leftover samples from this study on which you have participated in to be stored for use in future studies.
- After conducting test on the primary sample we believe some sample that may remain. That if stored could provide an opportunity to answer new questions or test old questions with new method
- Such findings in the near future would be useful in guiding policy towards clinical management of patient with cancer of the blood that shows up with persistent fever.

2. Procedure

- Samples will be kept under lock and key at very low temperature freezers available at MBN Clinical Laboratories, Nakasero Road, Kampala.
- Your confidentiality will be secured by use of only participant study ID/ Laboratory access numbers in place of your name.
- Samples will be de-identified for future studies
- After you have understood and agreed to allow your sample to be stored, you will be required to sign this sample storage consent form. A copy of which will be given to you.

3. Risks

No serious risks are expected from sample storage but if genetic testing is proposed as part of future studies. This could pose some risk. However since the samples will be de-identified, it will not be possible to link this information to corresponding participant.

4. Benefit

You may not directly benefit from this study however the information obtained will help guide the care providers in the management of patients that show up with persistently raised body temperature

5. Statement of voluntarism

Allowing your sample to be stored is voluntary. Even if you don't accept sample storage you can still participate in this study and you will not be penalized

6. Contacts for concerns/Questions:

If you have more questions and information that you may need clarifications on, please contact the Principle Investigator: **Ocung Guido** Tel **+256 754 736312 Email: guidoocung@gmail.com**

In case you have concerns regarding your rights as a participant, please feel free to contact the **Chairperson, School of Biomedical Sciences Higher Degrees Research & Ethics Committee [SBS-HDREC], Dr. Erisa Mwaka** Tel **+256 752575050** Email:**erisamwaka@yahoo.com**

7. Statement of Consent

I do acknowledge reading the information in this consent form or the information has been read and explained to me, I do understand the purpose of sample storage, what is going to be done, the risks, the benefits involved and my rights regarding study participation/consent withdraw, access to care, and confidentiality. Even if I do decline to sample storage, I can still continue to take part in the research study. Indicate your decision to this regard by ticking one of the boxes below.

☐ ***I agree to sample storage*** ☐ ***I disagree to sample storage***

---------- ---------- ----------

Name of participant (Print) Signature/thumbprint Date

For consents obtained for child participation.

☐ ***I agree to sample storage*** ☐ ***I disagree to sample storage***

_____

Name of the child (print).

---------- ---------- ----------

Name of caretaker (Print) Signature/thumbprint Date

Witness

By signing in this form, I do confirm that the information in this document was read to the participant/caretaker and she/he understands the purpose of sample storage, what is going to be done (steps to be undertaken), how confidentiality will be kept, the risks, the benefits involved and his/her rights regarding consent for sample storage. He/she has voluntarily consented for his/her (child's) sample to be stored for future use.

To those that use thumb prints for consent (Only):

I attest that the participant/ caretakers name is----------and has placed his/her thumbprint on this consent form on this date----------

Name of witness (Print)	Signature	Date
Name of study staff obtaining the consent (Print)	Signature	Date

APPENDIX III: ASSENT FORM

Assent forms in English. For use in all children volunteers aged 8 years and above.

Note: The assent will be administered to all eight (8) years of age and above, and from all persons incapable of self determination.

1. Brief Statement of study and purpose:

- You are being approached (requested) for your decision to take part in a research study that aims at checking for cytomegalovirus (a virus that causes fever besides other germs) in blood cancer patients with persistently raised body temperature.
- Therefore by knowing what cytomegalovirus is there, it will help guide the clinician to administer the most appropriate drug.

2. Procedure:

- If you accept to take part in this research study. I am going to ask your mother/caretaker a few questions about your name, age, where you stay and others.
- I will check your body to see if you have any illness. Two table spoonful of blood will be drawn from you for testing of cytomegalovirus using safe method.
- However to minimize piercing you again, the study sample will be taken off at the same time your doctor collects blood for your routine care
- A form used to record this information will be kept secret at all times.
- We would also like to know your HIV status so that you can be linked to a facility where you can access care in case you are found to be positive for HIV.

3. Voluntarism:

Enrollment into this study is voluntary and has no penalty incurred for your participation. You are free to decline to take part in this research study or withdraw from the study at any time and this will not affect your management.

4. Risks:

- You may experience some mild pain during blood draw and discomfort like bruises. However this will be minimized by ensuring that the study sample is taken off at the same time your doctor collects blood for your routine care using butterfly needles.

5. Confidentiality:

The results of this study will be kept strictly in secret, and used only for research purposes. A code will be used in place of your name for most of the study documents. Paper and computer records will be kept under lock and key and with password protection respectively. If the results of this research are published your names will not be shown.

6. Statement of Consent

I do confirm that the information in this assent form has been read and explained to the child as summarized in the form and that he /she has voluntarily accepted to participate in the study.

A copy of this assent form will be provided to the subject.

---------- ---------- ----------

Name of participant (Print) Signature/thumbprint Date

By signing in this form, I do confirm that the information in this assent form has been read to the child /subject and she /he understands the purpose of the study, the procedures and the fact that her/his participation in the study is voluntary and she /he has accepted to take part in the study.

---------- ---------- ----------

Name of parent/caretaker (Print) Signature/thumbprint Date

(To Assent process)

To those that use thumb prints for consent (Only):

I attest that the caretaker's name is ---------- and has placed his/her thumbprint on this consent form to allow the child called ---------- ---------- to participate in the study on this date----------.

Caretaker's relationship with the child ----------

---------- ---------- ----------

Name of witness (Print) Signature Date

---------- ---------- ----------

Name of study staff Signature Date

Obtaining Assent (Print)

APPENDIX IV: CARETAKERS INFORMED CONSENT FORM

Caretakers Informed Consent in English

1. Title of the proposed study:

Human Cytomegalovirus in febrile patients with underlying hematological malignancies at Uganda Cancer Institute

2. Investigator:

Ocung Guido, a Master student from Makerere University College of Health Sciences, Kampala

3. Introduction:

- You are being requested for your consent to allow the child under your care to take part in a research study
- This consent form gives you details about the research study. You can read it or it may be read to you and you are free to ask any question about anything that you may not understand.
- After you have understood and decided to allow your child take part in this research study, you will be required to sign this consent form. A copy of which will be given to you.
- The child's participation in this research study is voluntary. Even if he/she decides not to participate, he/she will still continue to receive care at the institute and will not be penalized.
- Upon signing this form, you will be asked a few questions, followed by body examination/checking of the child for any illness before 2 table spoonful of blood can be drawn from him/her for testing of cytomegalovirus (a virus that causes fever besides other germs)
- The child has a right to withdraw consent/assent for study participation at any time and for no reason.

4. Background and rationale for the study:

The reason this research study is being carried out is that, patients with cancer of the blood tend to have persistently raised body temperature despite adequate antibacterial/antifungal medication. Therefore by knowing what cytomegalovirus is there it can help guide the clinician to administer the most appropriate drug

5. Purpose of the study:

This study is being conducted in both children and adults with cancer of the blood that show up with a raised body temperature with the aim to find out how many of them have cytomegalovirus in their blood.

6. Study procedures:

- If the child agree to join the study, and after you have signed this consent form, your time in this study will last for only a few minutes.
- You will be asked a few questions concerning where you stay, the child's name, age, and others.
- The child will then be examined and blood samples collected from him/her for detection of cytomegalovirus germ using a safe method.
- A form used to record this information will be kept secret at all times.
- We would also like to know your child's HIV status so that the child can be linked to a facility where he/she can access care in case he/she is found to be positive for HIV.

7. Who will participate in the study?

161 children and adult patients with cancer of the blood that show up with raised body temperature upon voluntary consent will be studied

8. Risks/Discomforts:

- You may get some discomfort when you are being asked some questions. However you are free not to answer any question you may not be comfortable with.
- The child may experience some mild pain during blood draw and discomfort like bruises. However this will be minimized by ensuring that the study sample is taken off at the same time the child's doctor collects blood for your routine care.

9. Benefits:

The child may not directly benefit from this study however the information obtained will help guide care providers in the management of such patients that show up with persistently raised body temperature in the near future.

10. Alternatives:

The child does not have to take part in this research study if they do not want to. However he/she will still continue to receive medical care as before.

11. Cost:

There will be no costs to you as the child takes part in this research study.

12. Reimbursement:

The child will not be paid for study participation. However, **10,000/=** Ugandan shillings will be given as a contribution towards time spent while taking part in this study.

13. Contacts for concerns/Questions:
If you have more questions and information that you may need clarifications on, please contact the Principle Investigator: **Ocung Guido** Tel **+256 754 736312 Email: guidoocung@gmail.com**

In case you have concerns regarding child's rights as a participant, please feel free to contact the **Chairperson, School of Biomedical Sciences Higher Degrees Research & Ethics Committee [SBS-HDREC], Dr. Erisa Mwaka** Tel **+256 752575050** Email:**erisamwaka@yahoo.com**

14. Statement of voluntariness:

Enrollment into this study is voluntary and has no penalty incurred for the Childs participation. The child is free to decline to take part in this research study or withdraw from the study at any time and this will not affect his/her management.

15. Confidentiality:

The results of this study will be kept strictly in secret, and used only for research purposes. The child's identity will be kept secret and prevented from being known in as far as the law allows. A code will be used in place of child's name for most of the study documents. Paper and computer records will be kept under lock and key and with password protection respectively. If the results of this research are published, the child's names will not be shown.

16. What signing or putting a thumb print on the consent form means

You must understand that by signing this form, you do not waive any of your child's legal rights but merely indicate that you have been informed about the research study in which you have agreed your child to take part in. Signing often simply means that you have understood information in the consent form and thus accept your child to participate in the research study.

17. Statement of Consent
By signing in this form, I do confirm reading the information in this consent form or has been read to me and the child /subject, and she /he understands the purpose of the study, the procedures and the fact that her/his participation in the study is voluntary and she /he has accepted to take part in the study.

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Name of parent/caretaker (Print)	Signature/thumbprint	Date

(To Consent process)

To those that use thumb prints for consent (Only):

I attest that the caretaker's name is ---------- and has placed his/her thumbprint on this consent form to allow the child called ---------- ---------- to participate in the study on this date----------.

Caretaker's relationship with the child ----------

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Name of witness (Print)	Signature	Date
----------	----------	----------
Name of study staff obtaining the consent (Print)	Signature	Date

APPENDIX V: EKIWANDIIKO KYOKUKKIRIZA OKWETABA MU KUNOONYEREZA

1. Omutwe gwokunoonyereza:

Akawuka aka ‘Cytomegalovirus’ mu balwadde bomusujja abalina kookolo mu Uganda Cancer Institute.

2. Anoonyereza:

Ocung Guido, omuyizi ku daala elyokubiri mu tendekero lyebyasayansi erya setendekero ya Makerere, Kampala

3. Enyanjula:

- Otuukirirwa (osabibwa) kulwokukkirizakwo okwetaba mu kunoonyereza kuno
- Ekiwandiiko kyokukkiriza kino kikuwa ebikwata ku kunoonyereza. Osobola okukisoma oba nekikusomerwa era oliwaddembe okubuuza ekibuuzo kyonna ekikwata ku kintu kyonna kyoyinza obutategeera.
- Ngomaze okutegeera era ngosazeewo okwetaba mu kunoonyereza kuno, ojja kuweebwa ekiwandiiko kino oteekeko omukono. Ekiwandiiko kino kijja kukuweebwako.
- Okwetabakwo mu kunoonyereza kuno kwa kyeyagalire. Nebwoba osazeewo obuteetaba mu kunoonyereza ojja kusigala ngofuna endabilira okuva ku tendekero era tojja kutanzibwa.
- Ngomaze okuteeka omukono ku kiwandiiko kyokukkiriza kino, ojja kubuzibwayo ebibuuzo ebinagobelerwa okukebera omubili kulwobulwadde bwonna ng’omusaayi ogwenkanankana nobujiiko bwasukaali bubili tegunakujjibwako kulwokukebera akawuka ka ‘Cytomegalovirus’ (akawuka akaleetawo omusujja ngojeeko obuwuka obulala)
- Olina eddembe okujjamu okukkirizakwo kulwokwetaba mu kunoonyereza akadde konna nga tewaliwo nsonga.

4. Ebikwata ku kunoonyereza:

Ensonga lwaki okunoonyereza kuno kukolebwa, abalwadde ba kookolo womusaayi batera okubeera nebbugumu lyomubuli elyawaggulu newankubadde nga waliwo obujanjabi obumala obwokulwanyisa obuwuka. Nolwekyo okumanya kiki akawuka ka 'cytomegalovirus' kyekali awo kisobola okuyamba okulagilira abasawo okugaba eddagala elisinga okuba eddungi

5. Omugaso gwokunoonyereza:

Okunoonyereza kuno kukolebwa mu baana wamu nabantu abakulu abalina kookolo womumusaayi alabikira mu bbugumu lyomubili elilinye kulwekigendererwa kyokuzuula bameka kubo abalina akawuka ka 'Cytomegalovirus' mu musaayi.

6. Emitendera gyokunoonyereza:

- Bwokkiriza okwegata ku kunoonyereza, era ngomaze okuteeka omukono ku kiwandiiko kyokukkiriza kino, obuddebwo mu kunoonyereza kuno kujja kumala akadde katono.
- Ojja kubuuzibwayo ebibuuzo bitono ebikwata ku gyobeera, erinyalyo, emyaka nebilala
- Oluvanyuma ojja kukeberebwa era sampo yomusaayi ejja kukujjibwako kulwokukebera akawuka ka 'Cytomegalovirus' ngatukozesa enkola etalina bulabe.
- Foomu ekozesebwa okuwandiika obubaka buno ejjja kukuumibwa mu kyaama ebbanga lyonna.
- Era tujja kwagala okumanya embeerayo eyakawuka ka siliimu osobole okuyungibwa ku dwaliro lyoyinza okufunira obujanjabi singa osangibwa ngolina akawuka akaleeta mukenenya.

7. Ani aneetaba mu kunoonyereza?

Abaana 161 wamu nabantu abakulu abalina kookolo womumusaayi alabikila mu kulinya kwebbugumu lyomubili nga beyagalidde okukkiriza bajja kusomebwako

8. Akatyabaga/Ebitali bilungi:

- Oyinza okufunamu obutawulira bulungi ngobuuzibwa ebibuuzo ebimu, wabula oli waddembe obutayanukula kibuuzo kyonna kyoyinza obutayagala.

- Oyinza okufunamu obulumi butono mu biseera byokujako omusaayi wamu nokuwulira obubi okugeza ebikuyiro. Wabula kino kijja kukendeezebwa nga tukakasa nti sampolo yokunoonyereza ejjibwako mu kiseera kyekimu omusawowo wafunira omusaayi kulwendabilira eyabulijjo.

9. Emiganyuro:

Oyinza obutafuna mu kunoonyereza mbagilawo wabula obubaka obufunibwa bujja kuyamba okulagilira abalabilizi mu nzijanjaba yabalwadde abalabika mu kulinya kwebbugumu lyomubili mu biseera byomumaaso

10. Ebilala:

Toteekedwa kwetaba mu kunoonyereza kuno singa obeera toyagadde. Oyinza okusigala ngofuna enzijanjabayo nga bwekyali emabega

11. Ebisale

Tewaliwo bisale gyoli kulwokwetaba mu kunoonyereza kuno.

12. Okuddizibwa

Tojja kusasurwa kulwokwetaba mu kunoonyereza. Wabula, ojja kuweebwa omutwalo gumu ogwensimbi za Uganda ngokwongereza ku biserabyo kulwokwetabakwo mu kunoonyereza.

13. Endagiliro kulwebibuuzo:

Bwoba olina ebibuuzo wamu nobubaka bwewandiyagadde okutangaazibwako bambi tuukilira akulira okunoonyereza: **Ocung Guido** ku ssimu **+256 754 736312** omutimbagano gwa yintaneti: **guidoocung@gmail.com.**

Bwoba ngolina ensoga ezekuusa ku ddembelyo ngeyetabye mu kuunoonyeereza, bambi tuukirira sentebe wakakiiko kempisa nokunoonyereza akayitibwa '**School of Biomedical Sciences Higher Degrees Research & Ethics Committee [SBS-HDREC]', musawo Erisa Mwaka** ku ssimu **+256 752575050** omutimbagano gwa yintaneti:**erisamwaka@yahoo.com**

14. Olunyiriri lwokweyagalira:

Okuteekebwa mu kunoonyereza kuno kwa kyeyagalire era tekuliiko mutango kulwokwetabamukwo.Oliwaddembe okugaana okwetaba mu kunoonyereza oba okuvaamu akadde konna era kino tekijja kukosa ndabilirayo.

15. Okukuuma ebyama:

Ebinaava mu kunoonyereza kuno bijja kukuumibwa mu kyama era bikozeebwe kulwomugaso gwokunoonyereza kwokka. Ebikukwatako bijja kukuumibwa nobwekusifu era biziyizibwe okumanyibwa ngeteeka bwelikkiriza Namba yokunoonyereza ejja kukozesebwa mu kifo kyerinyalyo mu biwandiiko byokunoonyereza ebisinga. Ebiwandiiko byolupapula wamu ne kompyuta bijja kukuumibwa nekkufulu wamu nekisumuluzo ne pasiwaadi. Singa ebinaava mu kunoonyereza kuno binaafulumizibwa mu biwandiiko amanyago tegajja kulagibwa.

16. Kiki okuteeka omukono oba ekinkumu ku kiwanddiiko kyokukkiriza kyekitegeeza

Otekedwa okutegeera nti okuteeka omukono ku kiwandiiko kino, tokugira ddembelyo lya bwebanje wabula kilaga nti oteegeezedwa ku kunoonyereza kwokkirizza okweweyagalira okwetabamu. Okuteekako omukono kitegeeza nti otegedde obubaka mu kiwandiiko kyokukkiriza era nokkiriza okwetaba mu kunoonyereza.

17. Olunyiriri lwokukkiriza

Nkakasa okusoma obubaka obuli mu kiwandiiko kyokukkiriza kino oba obubaka bunsomedwa era nebunyinyonyorwa,ntegeera omugaso gwokunoonyereza, kiki ekigenda okukolebwa, akatyabaga, emiganyuro egilimu wamu neddembe lyange elyekuusa ku kwetabakwange/okukkiriza okuvaamu, okufuna obujanjabi, wamu nokukuuma ebyama era nzikirizza okwetaba mu kunoonyereza.

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Erinya lyeyetabyemu (Kyaapa) Omukono/ekinkumu Enaku zomwezi

Eri abo bokka abakozesa ekinkumu okukkiriza (Kyokka):

Nkakasa nti erinya lyeyetabyemu ye----------era atadde ekinkumukye ku kiwandiiko kyokukkiriza kino ku naku zomwezi----------

---------- ---------- ----------

Erinya lyomujulizi (Kyaapa) Omukono Enaku zomwezi

---------- ---------- ----------

Erinya lyanoonyereza afuna okukkiriza(Kyaapa) Omukono Enaku zomwezi

APPENDIX VI: EKIWANDIIKO KYOKUKKIRIZA OKUTELEKA SAMPOLO

Ekiwandiiko kyokukkiriza okutereka sampolo ezitakozesedwa okukozesebwa mu biseera byomumaaso.

1. Omugaso gwokuteleka sampolo

- Osabibwa okukkiriza sampolozo ezisigaddewo mu kunoonyereza kuno kwewetabyemu okutelekebwa zikozesebwe mu biseera byomumaaso.
- Oluvanyuma lwokukebera sampolo enkulu, tukkiriza nti sampolo ezimu ziyinza okusigala. Nti singa ziterekebwa kiyinza okuwa omukisa okwanukula ebibuuzo ebipya oba okugezesa ebibuuzo ebikadde nenkola empya.
- Ebinavaamu bijja kubeera byamugaso mu biseera byomumaaso mu kulagilira enkola ku kulabilira abalwadde abalina kookolo womumusaayi alagila mu musujja ogutawona

2. Emitendera

- Sampolo zijja kukuumibwa nekkufulu nekisumuluzo ku bbunyogovu bwawansi nyo mu matelekero agali ku labalatole za MBN Clinical laboratories, Nakasero Road, Kampala.
- Obwekusifubwo bujja kukuumibwa nga tukozesa namba yeyetabyemu yokka/oba namba za labalatole mu kifo kyerinyalyo.
- Sampolo zijja kujjibwako ebikukwatako kulwokunoonyereza kwebiseera byomumaaso
- Ngomaze okutegeera era nokkiriza sampolozo sitelekebwe, ojja kwetaagibwa okuteeka omukono ku kiwandiiko kino ekyokukkiriza okuteleka sampolo. Kopi yekiwandiiko ejja kukuweebwa.

3. Akatyabaga

Tewali katyabaga kamaanyi kasuubirwa naye singa okukebera obutonde kwetaagisa ngekitundu ku kunoonyereza kwebiseera byomumaaso. Kino kiyinza okuleetawo akatyabaga akatono. Wabula, engeri sampolo gyezinajibwako ebikukwatako, tekijja kuba kyangu kuyunga bubaka buno ku muntu yetabyemu.

4. Emiganyuro

Oyinza obutafuna mu kunoonyereza kwa mbagirawo wabula obubaka obufunibwa bujja kuyamba okulagilira abajanjabi mu kukwasaganya abalwadde abajja nebbugumu lyomubili elyawaggulu elitakoma

5. Olunyiriri lwokweyagalira

Okukkiriza sampolozo okutelekebwa kwa kyeyagalire. Nebwoba tokkiriza sampolozo kutelekebwa okyasobola okwetaba mu kunoonyereza era tojja kutanzibwa

6. Endagiliro kulwebibuuzo:

Bwoba olina ebibuuzo ebilala wamu nobubaka bwewandiyagadde okutangaazibwako bambi tuukilira akulira okunoonyereza: **Ocung Guido** ku ssimu **+256 754 736312 omutimbagano gwa yintaneti: guidoocung@gmail.com.**

Bwoba ngolina ensoga ezekuusa ku ddembelyo ngeyetabye mu kuunoonyeereza, bambi tuukirira sentebe wakakiiko kempisa nokunoonyereza akayitibwa '**School of Biomedical Sciences Higher Degrees Research & Ethics Committee [SBS-HDREC]', musawo Erisa Mwaka** ku ssimu **+256 752575050** omutimbagano gwa yintaneti:**erisamwaka@yahoo.com**

7. Olunyiriri lwokukkiriza

Nkakasa okusoma obubaka obuli mu kiwandiiko kyokukkiriza kino oba obubaka bunsomedwa era nebunyinyonyorwa, ntegeera omugaso gwokutereka sampolo, kiki ekigenda okukolebwa, akatyabaga, emiganyuro egilimu wamu neddembe lyange elyekuusa ku kwetabakwange/okukkiriza okuvaamu, okufuna obujanjabi, wamu nokukuuma ebyama. Nebwengaana okutereka sampolo, nkyasobola okugenda mu maaso nga netaba mu kunoonyereza Laga okusalawokwo kukino ngosaza ku kamu ku busanduuko wammanga.

☐ ***Nzikiriza okutereka sampolo*** ☐ ***Sikkiriza kutereka sampolo***

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Erinya lyeyetabyemu (Kyaapa) Omukono/ekinkumu Enaku zomwezi

Kulwokukkiriza okufunidwa kulwokwetabamu kwomwana.

☐ ***Nzikiriza okutereka sampolo*** ☐ ***Sikkiriza kutereka sampolo***

Erinya lyomwana (Kyaapa).

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Erinya lyomujanjabi (Kyaapa) Omukono/ekinkumu Enaku zomwezi

Omujulizi

Okuteeka omukono ku kiwandiiko kino, nkakasa nti obubaka mu kiwandiiko kino bwasomedwa eyetabyemu/ ajanjaba era ategeera omugaso gwokutereka sampolo, ekigenda okukolebwa (emitendera eginayitibwamu), butya obwekusifu gyebunakuumibwa,akatyabaga,emiganyuro egilimu wamu neddembelye mu kutereka onubaka.Yeyagalidde okukkiriza sampolo zomwanawe okutelekebwa kulwokukozesa mu biseera byomumaaso.

Eri abo bokka abakozesa ekinkumu okukkiriza (Kyokka):

Nkakasa nti erinya lyeyetabyemu oba abajanjabi be …….era atadde ekinkumukye ku kiwandiiko kyokukkiriza kino ku naku zomwezi----------

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Erinya lyomujulizi (Kyaapa) Omukono Enaku zomwezi

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Erinya lyanoonyereza afuna okukkiriza(Kyaapa) Omukono Enaku zomwezi

APPENDIX VII: EKIWANDIIKO KYABANA OKUKKIRIZA OKWETABA MU KUNOONYEREZA

Ekiwandiiko kyabaana okukkiriza okwetaba mu kunoonyereza abali wakati wemyaka munaana (8) nokudda waggulu.

Laba: Ekiwandiiko kino kijja kuweebwa abaana abali emyaka munaana nokudda waggulu, nabo abantu bonna abatasobola kwesalilawo.

1. **Olunyiriri olufunze olukwata ku kunoonyereza:**

   - Otuukirirwa (osabibwa) kulwokusalawo okwetaba mu kunoonyereza kuno okugenderera okukebera akawuka ka 'Cytomegalovirus' (akawuka akaleetawo omusujja ngojeeko obuwuka obulala) mu musaayi gwabalwadde ba kookolo abalina ebbugumu lyomubili elyawaggulu.

   - Nolwekyo okumanya kiki akawuka ka 'Cytomegalovirus' kye kiki, kijja kuyamba okulagilira omusawo okugaba eddagala elisinga obulungi.

2. **Emitendera:**

   - Bwokkiriza okwegata ku kunoonyereza, ngenda kubuuza mamawo ebibuuzo bitono ebikwata ku manyago, emyaka, gyobeera n'ebilala.

   - Njakukebera omubiligwo okulaba oba olina obulwadde. Omusaayi ogwenkanankana nobujiiko bwa sukaali bubili gujja kukujjibwako okukebera 'Cytomegalovirus' nga tukozesa enkola etalina bulabe.

   - Wabula okukendeeza ku kukufumita omulundi omulala, sampolo yokunoonyereza ejja kujjibwako mu kiseera kyekimu omusawowo wanakujjilako omusaayi kulwokujanjabakwo okwabulijjo

   - Foomu ekozesebwa okuwandiiko obubaka buno ejja kukuumibwa mu kyama ebbanga lyonna.

   - Era tujja kwagala okumanya embeerayo eyakawuka ka siriimu bweyimiridde okusobola okukukwasaganya nedwaliro wosobolera okufuna obujanjabi singa osangibwa ngolina obulwadde bwa siriimu

3. Olunyiriri lwokweyagalira:

Okuteekebwa mu kunoonyereza kuno kwa kyeyagalire era tekuliiko mutango kulwokwetabamukwo.Oliwaddembe okugaana okwetaba mu kunoonyereza oba okuvaamu akadde konna era kino tekijja kukosa ndabilirayo.

4. Akatyabaga/Ebitali bilungi:

- Oyinza okufunamu obulumi butono mu biseera byokujako omusaayi wamu nokuwulira obubi okugeza ebikuyiro.Wabula kino kijja kukendeezebwa nga tukakasa nti sampolo yokunoonyereza ejjibwako mu kiseera kyekimu omusawowo wafunira omusaayi kulwendabilira eyabulijjo nga tukozesa obuyiso bwekiwojjolo.

5. Okukuuma ebyama:

Ebinaava mu kunoonyereza kuno bijja kukuumibwa mu kyama era bikozeebwe kulwomugaso gwokunoonyereza kwokka. Namba yokunoonyereza ejja kukozesebwa mu kifo kyerinyalyo mu biwandiiko byokunoonyereza ebisinga. Ebiwandiiko byolupapula wamu ne kompyuta bijja kukuumibwa nekkufulu wamu nekisumuluzo wamu ne pasiwaadi. Singa ebinaava mu kunoonyereza kuno binaafulumizibwa mu biwandiiko amanyago tegajja kulagibwa.

6. Olunyiriri lwokukkiriza

Nkakasa okusoma obubaka obuli mu kiwandiiko kyabaana ekyokukkiriza kino era bunyinyonyodwa omwana nga bwebilambikidwa mu foomu era nti yeyagalidde okukkiriza okwetaba mu kunoonyereza.

Kopi yekiwadiiko kino ejja kuweebwa ku yetabyemu

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Erinya lyeyetabyemu (Kyaapa) Omukono/ekinkumu Enaku zomwezi

Okuteeka omukono ku kiwandiiko kino, nkakasa nti obubaka mu kiwandiiko kyabaana ekyokukkiriza busomedwa omwana/eyetabyemu era ategeera omugaso gwokunoonyereza, emitendera wamu nokuba nti okwetabamukwe kwa kyeyagalire era nti yeyagalidde okukkiriza okwetaba mu kunoonyereza

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Erinya lyomuzadde/ajanjaba (Kyaapa) Omukono/ekinkumu Enaku zomwezi (kulwokukkiriza kwomuto)

Eri abo bokka abakozesa ekinkumu okukkiriza (Kyokka):

Nkakasa nti erinya lyomujanjabi ye……era atadde ekinkumukye ku kiwandiiko kyokukkiriza kino okukkiriza omwanawe ayitibwa……..okwetaba mu kunoonyereza ku naku zomwezi……..

Oluganda lwomujanjabi ku mwana……..

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Erinya lyomujulizi (Kyaapa) Omukono Enaku zomwezi

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Erinya lyanoonyereza Omukono Enaku zomwezi

Afuna okukkiriza (Kyaapa)

APPENDIX VIII: OKUKKIRIZA KWAJANJABA OKWETABA MU KUNOONYEREZA

1. Omutwe gwokunoonyereza:

Akawuka aka ‘Cytomegalovirus’ mu balwadde bomusujja abalina kookolo mu Uganda Cancer Institute.

2. Anoonyereza:

Ocung Guido, omuyizi ku daala elyokubiri mu tendekero lyebyasayansi erya setendekero ya Makerere, Kampala

3. Enyanjula:

- Otuukirirwa (osabibwa) kulwokukkirizakwo omwana ali mu bulabilizibwo okwetaba mu kunoonyereza kuno
- Ekiwandiiko kyokukkiriza kino kikuwa ebikwata ku kunoonyereza. Osobola okukisoma oba nekikusomerwa era oliwaddembe okubuuza ekibuuzo kyonna ekikwata ku kintu kyonna kyoyinza obutategeera.
- Ngomaze okutegeera era ngosazeewo okukkiriza omwanawo okwetaba mu kunoonyereza kuno, ojja kuweebwa ekiwandiiko kino oteekeko omukono. Ekiwandiiko kino kijja kukuweebwako.
- Okwetaba kwomwanawo mu kunoonyereza kuno kwa kyeyagalire.Nebwoba osazeewo obuteetaba mu kunoonyereza ojja kusigala ngafuna endabilira okuva ku tendekero era tojja kutanzibwa.
- Nebwaba asazeewo obuteetabamu, ajja kusigala ngafuna obujanjabi okuva ku yinsitituti era tajja kutanzibwa
- Ngomaze okuteeka omukono ku kiwandiiko kyokukkiriza kino, ojja kubuzibwayo ebibuuzo , ebinagobelerwa okukebera omwana kulwobulwadde bwonna ng’omusaayi ogwenkanankana nobujiiko bwasukaali bubili tegunakujjibwako kulwokukebera akawuka ka ‘Cytomegalovirus’ (akawuka akaleetawo omusujja ngojeeko obuwuka obulala)
- Omwana alina eddembe okujjamu okukkirizakwe kulwokwetaba mu kunoonyereza akadde konna nga tewaliwo nsonga.

4. Ebikwata ku kunoonyereza:

Ensonga lwaki okunoonyereza kuno kukolebwa, abalwadde ba kookolo womusaayi batera okubeera nebbugumu lyomubuli elyawaggulu newankubadde nga waliwo obujanjabi obumala obwokulwanyisa obuwuka. Nolwekyo okumanya kiki akawuka ka 'cytomegalovirus' kyekali awo kisobola okuyamba okulagilira abasawo okugaba eddagala elisinga okuba eddungi

5. Omugaso gwokunoonyereza:

Okunoonyereza kuno kukolebwa mu baana wamu nabantu abakulu abalina kookolo womumusaayi alabikira mu bbugumu lyomubili elilinye kulwekigendererwa kyokuzuula bameka kubo abalina akawuka ka 'Cytomegalovirus' mu musaayi.

6. Emitendera gyokunoonyereza:

- Omwana bwakkiriza okwegata ku kunoonyereza, era ngomaze okuteeka omukono ku kiwandiiko kyokukkiriza kino, obuddebwo mu kunoonyereza kuno kujja kumala akadde katono.
- Ojja kubuuzibwayo ebibuuzo bitono ebikwata ku gyobeera, erinya lyomwanawo, emyaka nebilala
- Oluvanyuma omwana ajja kukeberebwa era sampo yomusaayi ejja kumujjibwako kulwokukebera akawuka ka 'Cytomegalovirus' ngatukozesa enkola etalina bulabe.
- Foomu ekozesebwa okuwandiika obubaka buno ejjja kukuumibwa mu kyaama ebbanga lyonna.
- Era tujja kwagala okumanya embeera yomwanawo eyakawuka ka siliimu asobole okuyungibwa ku dwaliro lyoyinza okufunira obujanjabi singa asangibwa ngolina akawuka akaleeta mukenenya.

7. Ani aneetaba mu kunoonyereza?

- Abaana 161 wamu nabantu abakulu abalina kookolo womumusaayi alabikila mu kulinya kwebbugumu lyomubili nga beyagalidde okukkiriza bajja kusomebwako

8. Akatyabaga/Ebitali bilungi:

- Oyinza okufunamu obutawulira bulungi ngobuuzibwa ebibuuzo ebimu,wabula oli waddembe obutayanukula kibuuzo kyonna kyoyinza obutayagala.

- Omwana ayinza okufunamu obulumi butono mu biseera byokujako omusaayi wamu nokuwulira obubi okugeza ebikuyiro.Wabula kino kijja kukendeezebwa nga tukakasa nti sampolo yokunoonyereza ejjibwako mu kiseera kyekimu omusawowo womwana wafunira omusaayi kulwendabilira eyabulijjo.

9. Emiganyuro:

Omwana ayinza obutafuna mu kunoonyereza mbagilawo wabula obubaka obufunibwa bujja kuyamba okulagilira abalabilizi mu nzijanjaba yabalwadde abalabika mu kulinya kwebbugumu lyomubili mu biseera byomumaaso

10. Ebilala:

Omwana tateekedwa kwetaba mu kunoonyereza kuno singa abeera toyagadde..Wabula ajja okusigala ngofuna enzijanjaba nga bwekyali emabega

11. Ebisale:

Tewaliwo bisale gyoli ng'omwana yetaba mu kunoonyereza kuno.

12. Okuddizibwa:

Omwana tajja kusasurwa kulwokwetaba mu kunoonyereza. Wabula, ajja kuweebwa omutwalo gumu ogwensimbi za Uganda ngokwongereza ku biserabye kulwokwetabakwe mu kunoonyereza.

13. Endagiliro kulwebibuuzo:

Bwoba olina ebibuuzo wamu nobubaka bwewandiyagadde okutangaazibwako bambi tuuukilira akulira okunoonyereza: **Ocung Guido** ku ssimu **+256 754 736312 omutimbagano gwa yintaneti: guidoocung@gmail.com.**

Bwoba ngolina ensoga ezekuusa ku ddembelyo ngeyetabye mu kuunoonyeereza, bamni tuukirira sentebe wakakiiko kempisa nokunoonyereza akayitibwa '**School of Biomedical Sciences Higher Degrees Research & Ethics Committee [SBS-HDREC]', musawo Erisa Mwaka** ku ssimu **+256 752575050** omutimbagano gwa yintaneti: **erisamwaka@yahoo.com**

14. Olunyiriri lwokweyagalira:

Okuteekebwa mu kunoonyereza kuno kwa kyeyagalire era tekuliiko mutango kulwomwana okwetabamu. Omwana waddembe okugaana okwetaba mu kunoonyereza oba okuvaamu akadde konna era kino tekijja kukosa ndabiliraye

15. Okukuuma ebyama:

Ebinaava mu kunoonyereza kuno bijja kukuumibwa mu kyama era bikozeebwe kulwomugaso gwokunoonyereza kwokka. Ebikwata ku mwana bijja kukuumibwa nobwekusifu era biziyizibwe okumanyibwa ngeteeka bwelikkiriza. Ebiwandiiko byolupapula wamu ne kompyuta bijja kukuumibwa nekkufulu wamu nekisumuluzo era ne pasiwaadi. Singa ebinaava mu kunoonyereza kuno binaafulumizibwa mu biwandiiko amanya gomwanawo tegajja kulagibwa.

16. Kiki okuteeka omukono oba ekinkumu ku kiwanddiiko kyokukkiriza kyekitegeeza:

Otekedwa okutegeera nti okuteeka omukono ku kiwandiiko kino, tokugira ddembe lyamwanawo lya bwebanje wabula kilaga nti oteegeezedwa ku kunoonyereza kwokkirizza omwanawo okweweyagalira okwetabamu. Okuteekako omukono kitegeeza nti otegedde obubaka mu kiwandiiko kyokukkiriza era nokkiriza omwanawo okwetaba mu kunoonyereza

17. Olunyiriri lwokukkiriza:

Okuteeka omukono ku kiwandiiko kino, nkakasa okusoma obubaka obuli mu kiwandiiko kyokukkiriza kino oba obubaka bunsomedwa era nebunyinyonyorwa wamu nomwana, era ategeera omugaso gwokunoonyereza, kiki ekigenda okukolebwa, wamu nukoba nti okwetabakwe mu kunoonyereza kwakyeyagalire era akkirizaa okwetabamu

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Erinya lyomuzadde/ajanjaba (Kyaapa) Omukono/ekinkumu Enaku zomwezi

(Kulwokukiriza okwetaba mu kunoonyereza)

Eri abo bokka abakozesa ekinkumu okukkiriza (Kyokka):

Nkakasa nti erinya lyajanjaba ye……..era atadde ekinkumukye ku kiwandiiko kyokukkiriza omwanawe ayitibwa……..kwetaba mu kunoonyereza kunaku zomwezi…….

Oluganda lwajanjaba nomwana ……

…… …… ……

Erinya lyomujulizi (Kyaapa) Omukono Enaku zomwezi

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Erinya lyanoonyereza afuna okukkiriza (Kyaapa) Omukono Enaku zomwezi

APPENDIX IX: CASE REPORT FORM (CRF)

BASELINE DATA

PARTICIPANTS INFORMATION.		
Participants study ID		
Inclusion/exclusion criteria	**Met all** ☐ **1**	**Not Met** ☐ **2**
Date of informed consent	D D M M M Y Y Y Y	Initials of study staff._____
Date of Birth	D D M M M Y Y Y Y	Estimated Age
Gender	**Male** ☐ **1**	**Female** ☐ **2**
Level of Education	**Primary** ☐ **Secondary** ☐ **Tertiary** ☐	
Type of accommodation, **Number of Occupants**	**Mud and wattle[ ] Semi-permanent [ ]** **Permanent[ ]** Number of Occupants:_____	
Address:	_____	
Intervention received by study participants.	**Chemotherapy regimen: […….]** [Tick the most appropriate option below] **Chemotherapy regime: Less Intensive [ ] Intensive[ ] (phase/cycle)** **Antibiotic use in the last 72 hours: Yes [ ] No[ ]** **Blood transfusion : Yes[ ] No[ ]** **HIV sero status: Pos[ ] Neg[ ] Unknown[ ]**	

Note: Please ensure that all the necessary fields have been filled in (ticked).

Participant study ID					

CLINICAL CHARACTERISTICS	LOW	HIGH
1. UNDERLYING HEMATOLOGICAL MALIGNANCY.	**Tick the most appropriate option below**	
• **Hodgkin lymphoma[HL]**	**No [ ]**	**Yes [ ]**
• **Non Hodgkin lymphoma[NHL]**	**No [ ]**	**Yes [ ]**
• **Acute myeloid leukemia[AML]**	**No [ ]**	**Yes [ ]**
• **Chronic myeloid leukemia[CML]**	**No [ ]**	**Yes [ ]**
• **Acute lymphoblastic leukemia[ALL]**	**No [ ]**	**Yes [ ]**
• **Chronic lymphocytic leukemia[CLL]**	**No [ ]**	**Yes [ ]**
• **Multiple myeloma**	**No [ ]**	**Yes [ ]**
• **Other possible coinfection, specify**	_____	
2. LATEST IMMUNOSUPPRESIVE TREATMENT.		
• **Monoclonal antibodies**	**No [ ]**	**Yes [ ]**
• **Steroid use within the last 1month**	**No [ ]**	**Yes [ ]**
LAB RESULTS from collected participant's blood sample.		
• **HCMV- IgG**	**Negative[ ]**	**Positive[ ]**
• **HCMV-IgM**	**Negative[ ]**	**Positive[ ]**
• **HCMV-DNA PCR**	**Negative[ ]**	**Positive[ ]**

Note: Please ensure that all the necessary fields have been filled in (ticked).

APPENDIX X: CHAIN OF CUSTODY FORM FOR RESEARCH STUDY SAMPLE SHIPMENT

Packaged at UCI by: _____ Date: _____ Time: _____

Transported to MBN by: _____ Date: _____ Time: _____

PARTICIPANT STUDY ID	SST	EDTA	Collected by	Date	Time	Storage Requested	Comment

Received at MBN by: _____ Date: _____Time: _____

Processed by: _____ Date: _____ Time_____