RESPONSE TO INDUCTION CHEMOTHERAPY IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA AT MOI TEACHING AND REFERRAL HOSPITAL, ELDORET

By

Ahoya Phinehas Ademi (MBChB)

SM/PGCHP/05/2010

Research thesis submitted to the School of Medicine in partial fulfilment of Master of Medicine degree in Child Health and Paediatrics, Moi University, School of Medicine

2014

RESPONSE TO INDUCTION CHEMOTHERAPY IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA AT MOI TEACHING AND REFERRAL HOSPITAL, ELDORET

By

Ahoya Phinehas Ademi (MBChB)

Moi University, School of Medicine

SM/PGCHP/05/2010

SUPERVISORS:

Dr. Constance N. Tenge

MBChB, MMed-Paediatrics

Senior Lecturer, Dept. of Child Health and Paediatrics

Moi University

Prof. Winstone Nyandiko M.

MBChB, MMed-Paediatrics; Cert Neonatology (BGU); MPH (HSPH)

Head, Dept. of Child Health and Paediatrics

Moi University

DECLARATION

1. Student's Declaration

This thesis is my original work and has not been presented for a degree in any other university. No part of this thesis may be reproduced without prior written permission of the author and or Moi University.

Dr. Ahoya Phinehas Ademi

Sign:…….. Date:……

2. Supervisors' Certification

This thesis has been submitted with our approval as university supervisors

Dr. Constance N. Tenge

Sign:……..... Date:……

Prof. Winstone Nyandiko M.

Sign:…….. Date:……

DEDICATION

I dedicate this research to my parents, Mr. and Mrs. Ademi, my beloved wife Mrs. Edna Ahoya and my dear children, Nicole, Ramona, Samantha and Elsie for their faith in me and their prayers to the Almighty.

ABSTRACT

Background
Acute leukemia is a common form of cancer in children comprising approximately 30% of all childhood malignancies in the developed countries. At The Moi Teaching and Referral Hospital (MTRH), acute lymphoblastic leukemia (ALL) is the second most common malignancy in the paediatric age group. Current cure rates in developed world approach 80%, while in the developing world they are less than 35%. This study looks at the response of paediatric ALL to induction chemotherapy which is a major factor in determining the likelihood of achieving cure.

Objective
To determine the response of paediatric acute lymphoblastic leukemia to induction chemotherapy at MTRH

Methodology
The Paediatric oncology unit in the paediatric ward, MTRH-Eldoret, Kenya was the study site. This was a Prospective Study design. The study subjects were children under the age of 14 years admitted to oncology ward who met study criteria. Demographic data and clinical features at presentation were documented together with the initial laboratory work-up in a data collection form. Bone Marrow Aspirate was done on 30 (100%) patients. On completion of induction, the clinical and laboratory responses together with the outcomes in terms of survival (remission or no remission) or death were documented. This data was stored in a password locked computer data base and analysis was performed using STATA version 12 special edition and presented in tables. Tests of association done by the Pearson's Chi Square test and survival analysis by Kaplan-Meier curves.

Results
Out of 30 patients, 20 (67%) were females and 20 (67%) were aged between 1-10years with the median age at enrolment being 8 years (IQR: 6-11). Patients who initially presented with anaemia were 28 (93%) and 25 (83%) of the patients presented with fever. Majority of the patients, 16 (53%) had a white blood cell count of above 50,000/ul. Only 1 (3%) of the patients had CNS disease at presentation. At completion of induction therapy, 23 (73%) patients went into complete remission (<5% blasts in bone marrow), 2 (7%) went into partial remission (5–25% blast cells in bone marrow) and 1 (3%) did not go into remission (>25% blast cells in the bone marrow). During the course of induction therapy, 4 (13%) patients died giving a case mortality rate of 3.4 deaths per 100 person days. There was significant association between mortality and patients aged less than 1 year (Fisher's exact P=0.020).

Conclusion

Most patients had good prognostic factors which include female gender, age between 1-10 years, no CNS disease at presentation and a white blood cell count less than 50,000/ul. Majority of these patients achieved complete remission. Patients below 1 year had bad outcomes. Long term studies need to be done for children with ALL for further survival analysis. More aggressive treatment is required for those presenting below the age of 1 year.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURE

ACKNOWLEDGEMENTS

I would like to appreciate Moi University, School of Medicine, The Moi Teaching and Referral Hospital for sponsorship.

I would sincerely like to thank my supervisors Dr. Tenge and Prof. Nyandiko for their guidance and contribution in this research thesis development.

I would also thank my colleagues and family for all the support during the research development.

Lastly I would like to acknowledge the biostatistician Mr. Keter A., for his input in this research.

LIST OF ABBREVIATIONS

ALL – Acute Lymphoblastic Leukemia

ALT – Alanine Transferase

AML – Acute Myelogenous Leukemia

BFM – Berlin-Frankfurt-Munster protocol

BMA – Bone Marrow Aspirate

BS for MPS – Blood slide for malaria parasites

CBC – Complete Blood Count

CCG – Children's cancer group

cGy - centi-Gray units

CI – Confidence Interval

CML – Chronic Myelogenous Leukemia

CNS – Central Nervous System

CXR – Chest X-Ray

EFS – Event Free Survival

FAB – French-American-British

Hb – Haemoglobin

HIV – Human Immunodeficiency Virus

IQR – Interquartile range

IREC – Institutional Research and Ethics Committee

IT – Intra-thecal

IV – Intravenous

Ksh. - Kenyan shillings

LBL – Lymphoblastic lymphoma

microL/ μL – micro-litre

mm - millimetres

MRD – Minimal Residual Disease

MTRH – Moi Teaching and Referral Hospital

OPD – Out patient department

PBF – Peripheral Blood Film

PGR – Prednisone Good Response

PPR – Prednisone Poor Response

REAL classification - Revised European-American Lymphoma classification

SCC – Sick child clinic

SEER - Surveillance, Epidemiology, and End Results

SOM – School of Medicine

SPSS – Statistical Package for the Social Scientists

TID – Thrice in a day

USA/US – United States of America

WHO – World Health Organisation

WBC – White Blood Cells

Yr. – year

OPERATIONAL DEFINITIONS

Child – defined in this context as any one below the age of 14 years.

Induction Chemotherapy – is the initial phase of cancer treatment and is designed to place the patient in remission.

Remission Induction – is the reduction of the number of lymphoblasts in a recovering bone marrow after induction chemotherapy. These blasts should be at least less than 5% in a Bone Marrow Aspirate and none in the peripheral blood film.

Remission - is defined by less than 5% blasts in the marrow with no blasts in the peripheral blood and a return of neutrophil and platelet counts to near-normal levels after 4–5 wk. of treatment.

Partial Remission – is having blasts in the marrow between 5% to 25% blasts in the bone marrow after induction chemotherapy.

No Remission – is having more than 25% blasts in the bone marrow after induction chemotherapy.

Paediatric ALL – Acute Lymphoblastic Leukemia (ALL) affecting children less than 14 years of age.

Event Free Survival – is used to show disease absent states over a period of time.

Minimal Residual Disease – is when residual leukemic cells remain following the achievement of "complete" remission, but the cells are below the limits of detection using conventional morphologic assessment. This can be evaluated using more sensitive assays and results in relapse.

Anaemia – haemoglobin level less than 8mg/dl (World Health Organisation moderate to severe anaemia definition)

Splenomegaly – palpable spleen more than 4 cm below left costal margin

Hepatomegaly – liver span of more than 11 cm or 4 cm below right costal margin

CHAPTER 1: INTRODUCTION

1.1 Background Information

Acute leukemia is the most common form of cancer in children comprising approximately 30% of all childhood malignancies[3]. Of these, acute lymphoblastic leukemia (ALL) occurs five times more commonly than acute myeloid leukemia.

In the United States of America, annual incidence of ALL is approximately 9-10 cases per 100,000 population in childhood[4].

Leukemia is the fourth most common paediatric malignancy in Kenya after Burkitt's lymphoma, nephroblastoma and Hodgkin's lymphoma[5]. In a study done in Kenyatta National Hospital (KNH) leukemia was the second most common childhood cancer[6]. In Moi Teaching and Referral (MTRH), ALL is the second commonest malignancy in the paediatrics.

ALL results from uncontrolled proliferation of immature lymphocytes. It has a striking peak incidence between 2–6 yr. of age and occurs more frequently in boys than in girls, at all ages.

The most common presenting symptoms of ALL are nonspecific (e.g., fever, bleeding, bone pain, lymphadenopathy). Signs of bone marrow failure are present with lymphadenopathy and hepato-splenomegaly. Unexplained persistence of any of these common signs or symptoms should prompt consideration of malignancy as a possible cause.

Diagnosis of ALL is achieved by determining the number of lymphoblasts in a bone marrow aspirate, more than 25% lymphoblasts being diagnostic.

In the Revised European-American Lymphoma classification (REAL) and World Health Organisation (WHO) classification systems for hematologic malignancies, the lymphoblastic neoplasm's, which may present as leukemia and/or lymphoma, are divided into two general categories based upon lineage:

- Precursor B-cell lymphoblastic leukemia/lymphoma, also called precursor B-cell ALL
- Precursor T-cell lymphoblastic leukemia/lymphoma (precursor T-LBL), also called precursor T-cell ALL

This is largely done because the prognosis and treatment differ between neoplasms of B and T cell lineage. These can be further divided into either lymphoblastic lymphoma or lymphoblastic leukemia.

Lymphoblastic lymphoma (LBL) is defined clinically if there is a mass lesion in the mediastinum or elsewhere and less than 25 percent blasts in the bone marrow. It is classified as ALL if there are more than 25 percent bone marrow blasts, with or without a mass lesion[1]. Within each lineage group, there is significant biological and clinical overlap between neoplasms' diagnosed as LBL and ALL. Although some patients present with predominantly lymphomatous involvement (e.g., a mediastinal mass or another defined lesion), most have or later develop marrow involvement. Similarly, patients who present with leukemia may have or develop extra medullary tumours. Accordingly, lymphoblastic lymphoma and acute lymphoblastic leukemia should be considered the same disease with different clinical presentations[2].

Before the use chemotherapy, this disease was fatal, usually within several months. The outcome of acute lymphoblastic leukemia in developing countries is inferior compared with the resource-rich nations. Current cure rates in developed world approach 80%[7, 8], while in the developing world they are less than 35%[9].

Successful treatment of ALL involves multi-drug regimen administration which is divided into several phases i.e. induction, consolidation and maintenance. Throughout this period, therapy directed to the Central Nervous System (CNS) is included. Most treatment protocols take 2-3 years to complete with specific regimens varying according to risk category.

Induction therapy is the initial phase of treatment and is designed to place the patient in remission. It is given with the primary goal of rapid restoration of bone marrow function, using multiple chemotherapy drugs at acceptable toxicities, in order to prevent the emergence of resistant sub-clones. Another goal is the use of adequate initial and prophylactic treatment of sanctuary sites such as the CNS, since CNS relapse is associated with a poor prognosis. Induction therapy aims to reduce the total body leukemia cell population from approximately $1x10^{12}$ cells to below the cytological detectable level of about $1x10^{9}$ cells.

More than 90 percent of children and adolescents[10, 11] with ALL enter clinical remission (CR) at the end of induction therapy regardless of their initial risk grouping. It involves weekly administration of vincristine for 3-4 weeks, daily corticosteroids (prednisone, prednisolone or dexamethasone) and asparaginase. A fourth agent such as an anthracycline (e.g. doxorubicin or daunorubicin) may be added to the three dose regimen, particularly for high risk patients. Intra-thecal cytarabine or methotrexate, or both should also be given. Other protocols have cytosar as part of IT medications if confirmed CNS involvement.

In MTRH the protocol for ALL therapy being used is quite similar to the best practices used in the developed world (see appendix III). It was adopted from the one in use at the Vrije University Hospital, Holland with assistance from the doctor 2 doctor foundation. Both protocols are adapted from the BFM protocol.

The MTRH protocol has L- asparaginase on it but the patients don't get it due to financial constraints. Lack of asparaginase makes our protocol inferior to the best practices.

The induction part starts with one week of prednisone and single dose of Intra-thecal methotrexate and hydrocortisone after tapping of CSF to check for CNS involvement. The IT is given on alternate weeks if no CNS disease is found or weekly if present with added cytosar. Five weekly doses of IV vincristine are given together with IV Adriamycin on week two and three. A BMA is done at week 6 to check for remission. The adaptation was done in consideration of presentation of the patients and availability of resources.

Induction failure is defined as more than 5% lymphoblasts in a recovering bone marrow aspirate after completion of induction therapy. Induction failure is an ominous sign.[12] The early response to therapy in the bone marrow is rated M1, M2 or M3. M1 represents a bone marrow aspirate displaying less than 5% residual leukemic blasts and signs of recovering haematopoiesis. M2 refers to a bone marrow aspirate with the presence of leukemic blasts in the range of 5% to 25%, while an M3 rating describes all bone marrow aspirates in which the percentage of leukemic blasts exceeds 25%.[14-16]

In this study, children were recruited after confirmed diagnosis of ALL, followed up and evaluated at the end of induction chemotherapy to check on remission status. This study was done to find out if the children with ALL in MTRH had good or bad outcomes after induction chemotherapy. It was to look for remission status so as to try and predict long term survival since this status can predict the Event Free Survival (EFS).

1.2 Problem Statement

ALL is one of the leading causes of cancer related mortality in developing countries. In the developed world it has the best prognosis with current 5-year survival rate being 88%[13]. In the developing world or resource limited countries the 5-year survival rates are less than 35%[9].

One of the factors of good prognosis is the outcome achieved from induction chemotherapy[7]. Slow responders after induction chemotherapy having a high risk of relapse and those with no remission have a dismal prognosis[59, 60].

In the developed countries, stratified management of children with ALL is done during induction chemotherapy so as to achieve remission.[55] This might be the reason why they have good prognosis and better cure rates than the developing countries.

In MTRH, children with ALL get a standard protocol with no stratification according to risk factors. A study on the outcome of induction chemotherapy will assist in finding out if stratification of these children is required. Further analysis of the outcomes compared with prognostic indicators will also assist in judging if optimal care is given to these children.

We do not have the concrete information about the presentations and outcomes of children in resource limited settings after induction chemotherapy. There are no studies on outcomes in Sub-Saharan Africa. This makes it difficult for stratified management. Therefore, with no stratified management of these children, developing countries might not achieve survival rates as the developed countries.

CHAPTER 2: LITERATURE REVIEW

2.1 Epidemiology and Classification of ALL

ALL is the most common childhood malignancy in the developed world, while in the developing world, the incidence and prevalence is rising[19].

Childhood ALL was one of the first disseminated cancer shown to be curable and consequently has represented the model malignancy for the principles of cancer diagnosis, prognosis, and treatment. It is a heterogeneous group of malignancies with a number of distinctive genetic abnormalities that result in varying clinical behaviours and responses to therapy.

Approximately 2500 to 3500 children are diagnosed with ALL in the USA each year with an incidence of 2.8 cases per 100,000. It has a striking peak incidence between 2–6 yr. of age and occurs more frequently in boys than in girls, at all ages[17-18]. The rate in US whites is higher than that of US Blacks, and the rates in the Hispanic subgroup are the highest of all.[3]

The incidence appears to be increasing as shown by a study from Great Britain whereby the incidence of leukemia (mostly attributable to ALL) has steadily increased from 3.83 to 4.61 per 100,000 persons by sex and age from the five-year period of 1971 to 1975 and 1996 to 2000.[19]

In a prospective study done in Kenya by Mwanda OW, from June 1997 to December of the same year found that ALL is the fourth prevalent cancer in children younger than 16 years with a prevalence of 7.6%. In this study 157 cases were evaluated from seven provincial hospitals and KNH.[5]

In an earlier hospital based study of childhood cancers, it showed leukemia as the second most cancer with the frequency of ALL appearing to be increasing as compared to much earlier studies.[6]

The disease is more common in children with certain chromosomal abnormalities, such as Down syndrome, Bloom syndrome, Neurofibromatosis type 1, ataxia-telangiectasia, and Fanconi syndrome.[20-21] Among identical twins, the risk to the second twin if one develops leukemia is greater than that in the general population. The risk is greater than 70% if the first twin is diagnosed during the first year of life and the twins shared the same (monochorionic) placenta. If the first twin develops ALL by 5–7 yr. of age, the risk to the second twin is at least twice that in the general population, regardless of zygosity. An increased risk of ALL associated with higher birth weight has also been noted in a study done in the Nordic countries.[22]

In virtually all cases, the aetiology of ALL is unknown, although several genetic and environmental factors are associated with childhood leukemia. Exposure to medical diagnostic radiation both in utero and in childhood has been associated with an increased incidence of ALL. In addition, published descriptions and investigations of geographic clusters of cases have raised concern that environmental factors may increase the incidence of ALL. Thus far, no such factors other than radiation have been identified in the USA. In certain developing countries, there has been an association between B-cell ALL and Epstein-Barr viral infections.

Studies of the relationship between childhood ALL, urban/rural status and population density, as well as other possible etiologic factors (e.g., environmental exposures, abnormal immune response to common infection(s) have yielded inconsistent results.[23-25]

ALL classification depends on characterizing the malignant cells in the bone marrow to determine the morphology, phenotypic characteristics as measured by cell membrane markers, and cytogenetic and molecular genetic features.

Morphologic classification of ALL often is based on evaluation of the bone marrow aspirate using the French-American-British (FAB) system.[26] According to this system: L1 lymphoblasts are small cells with scant cytoplasm, condensed nuclear chromatin, and indistinct nucleoli. Most children with ALL cases (85% to 89%) are classified as having FAB L1.[27, 28] L2 lymphoblasts are larger cells with a moderate amount of cytoplasm, dispersed chromatin, and multiple nucleoli. In some studies, L2 has been associated with worse prognosis than has L1.[27, 29] However, when patients are stratified according to age, sex, and initial WBC, differences in prognosis between L1 and L2 are no longer observed.[28] Eleven to 14% of cases of ALL in children are classified as FAB L2.[27, 28] L3 lymphoblasts, also known as Burkitt leukemia, have deep cytoplasmic basophilia with prominent cytoplasmic vacuolation. L3 morphology correlates with a more guarded prognosis. The L3 cell usually has mature B-cell characteristics and often is treated using drugs effective for highly aggressive B-cell lymphoma variants. Less than 1% of cases of ALL in children are classified as FAB L3.[27, 28] Morphology alone usually is adequate to establish a diagnosis, but the other studies are essential for disease classification, which may have a major influence on both the prognosis and the choice of appropriate therapy.

Phenotypically, surface markers show that about 70% to 80% of cases of ALL are derived from progenitors of B cells, about 15% to 17% are derived from T cells, and about 1% is derived from B cells. B-precursor leukemia typically is CD10+, CD19+, and sometimes CD20+. Leukemic lymphoblasts with the L3 morphology usually have

markers for mature-B-cell ALL (CDs 10± 19,20,22,25, and surface immunoglobulin). Cases of precursor T lymphoblastic leukemia are positive for CDs 2, 3, 4, 5, 7, and 8. A small percentage of children diagnosed with leukemia have a disease characterized by surface markers of both lymphoid and myeloid derivation. This is the reason why immunophenotyping should not be used as the sole means of leukemia classification.[30] Immunophenotypes often correlate to disease manifestations.

Chromosomal abnormalities are found in most patients with ALL. The abnormalities, which may be related to chromosomal number, translocations, or deletions, provide important prognostic information[31, 32]. Children with lymphoblasts exhibiting hyperdiploidy (54 to 58 chromosomes) have the best prognosis, particularly if associated with the combined trisomies of chromosomes 4, 10, or 17[33, 34]. The unfavourable structural chromosomal abnormalities are the translocations t(4;11), t(1;19), t(8;14), t(1;14) and t(9;22)[35-39]. Extreme hyperdiploidy (59 to 84 chromosomes) or hypodiploidy (fewer than 45 chromosomes) is associated with poor outcome[40, 41]. More aggressive treatment regimens are recommended for children with any of the unfavourable structural chromosomal abnormalities. Patients with t(1;19) respond well to such strategies, whereas the addition of tyrosine kinase inhibitor therapy should be considered in patients with t(9;22). Some structural abnormalities are associated with favourable prognosis. They include the t(12;21) rearrangement in B-precursor ALL, which occurs in 20 to 25 percent of cases of childhood ALL[42, 43].

In addition, one study showed favourable outcome in T cell ALL patients with t(10;14)[44]. The polymerase chain reaction and fluorescence in situ hybridization techniques, for example, offer the ability to pinpoint molecular genetic abnormalities and to detect small numbers of malignant cells during follow-up; however, the clinical

utility of these findings has yet to be firmly established. The development of DNA microanalysis makes it possible to analyse the expression of thousands of genes in the leukemic cell. This technique promises to further enhance the understanding of the fundamental biology and to provide clues to the therapeutic approach of ALL.

2.2 Clinical Manifestations

The initial presentation of ALL usually is nonspecific and relatively brief. Anorexia, fatigue, and irritability often are present, as is an intermittent, low-grade fever. Bone or, less often, joint pain, particularly in the lower extremities, may be present. Bone pain, particularly affecting the long bones, and caused by leukemic involvement of the periosteum, is a presenting symptom in 21 to 38 percent of cases of acute leukemia[45]. Young children with such pain may present with limp or refusal to bear weight[30]. Patients often have a history of an upper respiratory tract infection in the preceding 1–2 mo. Less commonly, symptoms may be of several months' duration, may be localized predominantly to the bones or joints, and may include joint swelling. Bone pain is severe and may wake the patient at night. As the disease progresses, signs and symptoms of bone marrow failure become more obvious with the occurrence of pallor, fatigue, bruising, or epistaxis, as well as fever, which may be caused by infection.

Although uncommon (less than 5% of cases), leukemia involving the CNS can present with symptoms of increased intracranial pressure, including headache, vomiting, lethargy, and/or nuchal rigidity[46]. Others include papilledema, retinal haemorrhages, and cranial nerve palsies. Rarely, leukemia can present with cranial nerve abnormalities[47].

On physical examination, findings of pallor, listlessness, purpuric and petechial skin lesions, or mucous membrane haemorrhage may reflect bone marrow failure. The proliferative nature of the disease may be manifested as lymphadenopathy, splenomegaly, or, less commonly, hepatomegaly. Approximately 50 percent of children with ALL present with lymphadenopathy, which is one of the indications of extra medullary leukemic spread[30]. As a general rule, a lymph node is considered enlarged if it is greater than 10 mm in its greatest diameter. Exceptions to this rule include the following: Epitrochlear nodes are enlarged if they are greater than 5 mm; inguinal nodes are enlarged if they are greater than 15 mm in greatest diameter; and cervical nodes are enlarged if they are greater than 20 mm.

In patients with bone or joint pain, there may be exquisite tenderness over the bone or objective evidence of joint swelling and effusion. Nonetheless, with marrow involvement, deep bone pain may be present but tenderness will not be elicited. Bone pain also results from aseptic osteonecrosis because of malignant cell necrosis in the bone marrow involvement[48]. Respiratory distress usually is related to anaemia but may occur in patients with an obstructive airway problem due to a large anterior mediastinal mass (e.g., in the thymus or nodes). This problem is most typically seen in adolescent boys with T-cell ALL. T-cell ALL also has a higher leukocyte count.

The median leukocyte count at presentation is 33,000, although 75% of patients have counts more than 20,000; thrombocytopenia is seen in 75% of patients, and hepatosplenomegaly is seen in 30–40% of patients. Approximately 50 percent of children have WBC counts less than 10,000/microL and 20 percent have an initial leukocyte count more than 50,000/microL[30]. Approximately one-half of children with ALL present with bleeding (including petechiae and purpura) and three-quarters have a

platelet count less than 100,000/microL at the time of diagnosis[30]. Testicular (20%) and ovarian (30%) involvement occurs but does not require a biopsy.

2.3 Diagnosis

The ALL diagnosis is suggested by peripheral blood findings indicative of bone marrow failure. Bone marrow examination should be performed for the following indications: Atypical cells in the peripheral blood; unexplained depression of more than one peripheral blood element; and unexplained lymphadenopathy or hepatosplenomegaly associated with cytopenias. Anaemia and thrombocytopenia are seen in most patients. Leukemic cells often are not observed in the peripheral blood in routine laboratory examinations. Many patients with ALL present with total leukocyte counts of less than 10,000/μL. In such cases, the leukemic cells often are reported initially to be atypical lymphocytes, and it is only on further evaluation that the cells are found to be part of a malignant clone.

When the results of an analysis of peripheral blood suggest the possibility of leukemia, a bone marrow examination should be done to establish the diagnosis. BMA alone usually is sufficient, but sometimes a bone marrow biopsy is needed to provide adequate tissue for study or to exclude other possible causes of bone marrow failure.

ALL is diagnosed by a bone marrow evaluation that demonstrates greater than 25% of the bone marrow cells as a homogeneous population of lymphoblasts. Staging of ALL is based partly on CSF examination. If lymphoblasts are found and the CSF leukocyte count is elevated, overt CNS or meningeal leukemia is present. This finding reflects a worse stage and indicates the need for additional CNS and systemic therapies. The staging lumbar puncture may be performed in conjunction with the first dose of

intrathecal chemotherapy if the diagnosis of leukemia has been previously established from bone marrow evaluation.

2.4 Treatment

Without effective therapy, ALL is fatal. Combination chemotherapy is the primary treatment modality for patients with this disease. The survival rates of children with ALL over the past 40 yr. have improved as the results of clinical trials have improved the therapies and outcomes. There is no single best regimen for induction therapy in ALL and patients should be encouraged to participate in clinical trials whenever possible.

The outcomes of cancer are in form of event-free survival rates. EFS rates for ALL have steadily improved since the 1980s. The overall five-year EFS for childhood ALL currently over 80 percent in the developed world and the 10-year EFS is about 60 percent [7]. The 5 and estimated 10-year survival rates for patients diagnosed from 2000 to 2004 were 88 and 84 percent, respectively [13].

The 10-year survival rate for children diagnosed in the 2005 to 2009 period is estimated to be 88 percent.

In contrast, in the developing world, cure rates are less than 35 percent [9], in part because of abandonment of treatment [49] and/or lack of dedicated, multidisciplinary paediatric oncology units [50]. The median survival period is now more than 10 years overall, the 5-year survival rate remains poor for Black males under 4 years of age. Socioeconomic factors do not account for this difference, which may relate to ALL subtype distribution.[3]

The choice of treatment of ALL is based on the estimated clinical risk of relapse in the patient, which varies widely among the subtypes of ALL. Three of the most important predictive factors are the age of the patient at the time of diagnosis, the initial leukocyte count, and the speed of response to treatment (i.e., how rapidly the leukemic cells can be cleared from the marrow or peripheral blood). Different study groups use various factors to define risk, but age between 1–10 yr. and a leukocyte count of less than 50,000/μL are widely used to define average risk. Children who are over 10 yr. of age or who have an initial leukocyte count of more than 50,000/μL are considered to be at higher risk. The outcome for patients at higher risk can be improved by administration of more intensive therapy despite the greater toxicity of such therapy. Infants with ALL, along with patients who present with specific chromosomal abnormalities such as t(9;22) or t(4;11), have an even higher risk of relapse despite intensive therapy.

Clinical trials have demonstrated that the prognosis for patients with a slower response to initial therapy may be improved by therapy that is more intensive than the therapy considered necessary for patients who respond more rapidly. Most children with ALL are treated on clinical trials conducted by national or international cooperative groups.

In general, the initial therapy is designed to eradicate the leukemic cells from the bone marrow. During this phase, therapy usually is given for 4 wk. and consists of vincristine weekly, a corticosteroid such as dexamethasone or prednisone, and either repeated doses of native L-asparaginase or a single dose of a long-acting, pegylated asparaginase preparation. Intrathecal cytarabine or methotrexate, or both, also may be given. Patients at higher risk also receive daunomycin at weekly intervals. With this approach, 98% of patients are in remission, as defined by <5% blasts in the marrow and a return of neutrophil and platelet counts to near-normal levels after 4–5 wk. of treatment.

Intrathecal chemotherapy is usually given at the start of treatment and once more during induction.

The second phase of treatment focuses on CNS therapy in an effort to prevent later CNS relapses. Intrathecal chemotherapy is given repeatedly by lumbar puncture in conjunction with intensive systemic chemotherapy. The likelihood of later CNS relapse is thereby reduced to less than 5%. A small proportion of patients with features that predict a high risk of CNS relapse may receive irradiation to the brain and spinal cord. This includes those patients who, at the time of diagnosis, have lymphoblasts in the CSF and either an elevated CSF leukocyte count or physical signs of CNS leukemia, such as cranial nerve palsy.

After remission has been induced, many regimens provide 14–28 wk. of multiagent therapy, with the drugs and schedules used varying depending on the risk group of the patient.

Finally, patients are given daily mercaptopurine and weekly methotrexate, usually with intermittent doses of vincristine and a corticosteroid. This period, known as the maintenance phase of therapy, lasts for 2–3 yr., depending on the protocol used. Many patients benefit from administration of a delayed intensive phase of treatment (delayed intensification), approximately 5–7 mo. after the beginning of therapy. This is after a relatively nontoxic phase of treatment (interim maintenance) to allow recovery from the initial intensive therapy. A small number of patients with particularly poor prognostic features, principally those with the t(9;22) translocation known as the Philadelphia chromosome, may undergo bone marrow transplantation during the first remission. In ALL, this chromosome is similar but not identical to the Philadelphia chromosome of CML.

Treatment also may be stratified by gene expression profiles of leukemic cells. In particular, gene expression arrays induced by exposure to the chemotherapeutic agent can predict which patients have drug-resistant ALL. Furthermore, pharmacogenetic testing of the thiopurine S-methyltransferase gene, which converts mercaptopurine or thioguanine (both prodrugs) into active chemotherapeutic agents, can identify rapid metabolizers (associated with toxicity) or slow metabolizers (associated with treatment failure), thus optimizing drug dosing.

The major impediment to a successful outcome is relapse of the disease. Relapse occurs in the bone marrow in 15–20% of patients with ALL and carries the most serious implications, especially if it occurs during or shortly after completion of therapy. Intensive chemotherapy with agents not previously used in the patient followed by allogeneic stem cell transplantation can result in long-term survival for a few patients with bone marrow relapse.

Patients with relapse in the CNS usually present with signs and symptoms of increased intracranial pressure and may present with isolated cranial nerve palsies. The diagnosis is confirmed most readily by demonstrating the presence of leukemic cells in the CSF and, rarely, by imaging studies. The treatment includes intrathecal medication and craniospinal irradiation. Systemic chemotherapy also must be used, because these patients are at high risk for subsequent bone marrow relapse. Most patients with leukemic relapse confined to the CNS do well, especially those in whom the CNS relapse occurs after chemotherapy has been completed or during the latter phase of chemotherapy.

Testicular relapse occurs in 1–2% of boys with ALL, usually after completion of therapy. Such relapse presents as painless swelling of one or both testes. The diagnosis

is confirmed by biopsy of the affected testis. Treatment includes systemic chemotherapy and local irradiation. A high proportion of boys with a testicular relapse can be successfully re-treated, and the survival rate of these patients is good.

Close attention to the medical supportive care needs of the patients is essential in successfully administering aggressive chemotherapeutic programs. Patients with a large tumour burden are prone to tumour lysis syndrome as therapy is initiated. The renal failure associated with very high levels of serum uric acid can be prevented with the use of urate oxidase. Chemotherapy often produces severe myelosuppression, which may require erythrocyte and platelet transfusion and which always requires a high index of suspicion and aggressive empirical antimicrobial therapy for sepsis in febrile children with neutropenia. Patients must receive prophylactic treatment for *Pneumocystis* pneumonia during chemotherapy and for several months after completing treatment.

In a study done to determine the pattern of deaths in children with ALL treated at a single center in India and to identify the problem areas in management, case records of 532 patients with ALL were analyzed. One hundred twenty-eight (24.0%) deaths were recorded. Sepsis (53.3%) and bleeding (15.7%) were the most common causes of mortality. The factors associated with an increased risk of death were longer symptom diagnosis interval (P=0.049), bulk disease (P=0.008), mediastinal adenopathy (P=0.001), higher total leukocyte count (P=0.001), and lower platelet count (P=0.007) at presentation as compared with the survivors. Multivariate analysis showed that longer symptom diagnosis interval (P=0.001), mediastinal adenopathy (P=0.006), lower platelet count (P=0.001), and higher total leukocyte count significantly influenced death. The estimated median time to death for the induction and remission deaths were 0.5 and 17 months, respectively[51].

The success of therapy has changed ALL from an acute disease with a high mortality rate to a chronic disease. However, such chronic treatment can incur substantial academic, developmental, and psychosocial costs for children with ALL and considerable financial costs and stress for their families. Because of the intensity of therapy, long-term and acute toxicity effects may occur. An array of cancer care professionals with training and experience in addressing the myriad of problems that may arise is essential to minimize the complications and achieve an optimal outcome.

2.5 Prognosis.

Most children with ALL can now be expected to have long-term survival, with the survival rate greater than 80% at 5 yr. from diagnosis. The most important prognostic factor is the choice of appropriate risk-directed therapy, with the type of treatment chosen according to the type of ALL, the stage of disease, the age of the patient, and the rate of response to initial therapy (favourable if the patient responds in less than 1 mo.). Characteristics generally believed to adversely affect outcome include age less than 1 yr. or greater than 10 yr. at diagnosis, a leukocyte count of greater than 100,000/μL at diagnosis, or a slow response to initial therapy.

Chromosomal abnormalities, including hypodiploidy, the Philadelphia chromosome, and MLL gene rearrangements and translocations [t(1:19) or t(4;11)], portend a poorer outcome. More favourable characteristics include a rapid response to therapy, hyperdiploidy, trisomy of specific chromosomes, and rearrangements of the TEL/AML1 genes.

Minimal residual disease (MRD) can be detected with specific molecular probes to translocations and other DNA markers contained in leukemic cells. MRD can be

quantitative and can provide an estimate of the burden of leukemic cells present in the marrow. Although it is not known how much MRD can be eliminated by the patient's immune host defence mechanisms, an elevated degree of MRD present at the end of induction suggests a poor prognosis and a strong possibility of relapse.

A study done to compare the prognostic determinants in childhood ALL between Negroid and Caucasian populations showed that there was a statistically significant higher population of children with high total WBC count in African children compared to developed countries.

Proportions for other factors in developed countries and developing countries respectively were as follows: T-cell immune-phenotype (17% and 60%), FAB-L2, L3 (15% and 83%), CNS involvement (5% and 13%) and mediastinal shadows on CXR (8% and 13%). This study concluded that the frequency of poor prognostic determinants for ALL in developing African countries is higher than in developed countries. It further recommend basic epidemiologic research on childhood ALL in African countries[52].

Induction remission is one of the factors that determine cure rates of ALL. Good induction remission is shown by having less than 5% lymphoblast in the bone marrow aspirate done after induction phase of chemotherapy.

In one study of 774 children with ALL from the United States, 23 had persistent leukemia after completion of induction therapy and were treated with additional induction chemotherapy. Although 21 patients eventually achieved remission, their five-year event-free survival (EFS) was only 16 percent (95% CI: 0-31 percent) regardless of management regimen, compared to 82 percent (95% CI: 79-86 percent) in

the group that achieved remission within one month and 79 percent (95% CI: 70-87 percent) in those who had protracted hypoplasia.[53]

Another study aimed at analysis whether there is any association between the long-term EFS in patients with good and poor response to prednisone, which is used in the induction course of ALL. The study group consisted of 179 children, aged 2 to 17 years, qualified into low and medium risk groups, treated according to the BFM-86 and BFM-90 protocols in the Department of Paediatrics and Haematology of Silesian Centre of Paediatrics in Zabrze between 1986 and 1996. In the study group, 89.9% (161) of children showed good prednisone response (PGR) as compared to 10.1% of children with poor response to steroids (PPR).

Cumulative probability of 15-year EFS for the PGR group was 70% and significantly differed from EFS in PPR group (39%; $p = 0.006$). In the study group, the children showing good response to the initial treatment with prednisone have higher chance for durable remission and subsequent cure.[54]

In a French study of 1395 children with ALL, 53 patients failed induction therapy. With salvage therapy, 43 patients entered complete remission, 39 after one second-line course of chemotherapy and four who required more than one course. Both the overall survival rate and the EFS for the 53 patients who failed induction therapy compared to those who responded to therapy were markedly lower (30 and 27 percent versus 85 and 75 percent, respectively).[10]

In a study on the determinants of outcome after intensified therapy of childhood lymphoblastic leukemia in the United Kingdom, multivariate analysis showed that remission status at end of induction therapy was one of the significant predictors of

outcome. Study was done on 2090 children with treatment allocation not determined by risk factors.[55]

In a descriptive study conducted at paediatric Oncology Department of Children Hospital Complex Multan, Pakistan, from December 2005 to December 2008; Out of 38 Patients studied, 26 (68%) were males. Age range was between 2–12 years (Mean 5.4 years). Bone Marrow Biopsy was done in 38 (100%) and Immunophenotyping in 34 (89%) patients. At day 28 of induction therapy, 28 (74%) patients went into complete remission (<5% blast cells in bone marrow), 2 (5%) into partial remission (5–25% blast cells in bone marrow) and 1 (3%) was not in remission (>25% blast cells in the bone marrow). Seven (18%) patient died due to febrile neutropenia and sepsis during the course of induction therapy.[56]

Another study done in Karachi, Pakistan, children diagnosed with ALL were evaluated over a period of 17 years (January 1, 1989 to December 31, 2006). They found that 46 patients were diagnosed during the study period and were on regular follow-up. Forty five (97.8%) of these were in complete remission after 28 days of induction therapy. Thirty patients (65.2%) were alive and doing well at the time of study. Of these 30 patients, 26 (86.6%) remained relapse free while only four (13.3%) had relapsed. The remaining 16 patients (34.7%) did not survive including 11 (68.7%) who had a relapse. The only significant variables in terms of prognosis were age and ALL phenotype with a P value 0.04 and 0.03 respectively.[57]

In a retrospective descriptive study conducted at Aga Khan University Hospital, Karachi - Pakistan, comprising data related to children below 15 years of age and treated between January 1997 and December 2006, found of the total 121 children diagnosed with the condition, 79 (65.3%) were males; 86 (71.1%) patients were

between 1-9 years of age. Immunophenotyping was done in 99 (81.81%) patients: 86 (87%) cases had precursor B and 13 (13.13%) had precursor T. Of the total, 106(87.6%) patients opted for treatment, while 15 (11.6%) were lost to follow-up. Besides, 26(21.7%) patients had at least one relapse; the most common site being bone marrow in 13 (50%) followed by central nervous system in 9 (36.6%). There were 20(16.5%) deaths in the sample. Infection was the most frequent cause of death. The event-free survival and overall survival was 63% (n=76) and 65% (n=79) respectively.[58]

An older study done by Macdougall LG, et al [62] in 1986 on acute childhood leukemia in Johannesburg, they found that remission rates for black children with ALL were lower than for white children and the cumulative proportion of black patients surviving at 60 months was only 32% compared with 72% of white patients ($p = 0.0001$).

A much recent study (2011) done in Mali where only 12 cases of childhood lymphoblastic leukemia were recruited, they found an overall survival rate of 66.7% at 5 years of follow-up.[63]

As shown in this literature search, studies on children with ALL in Africa are few and far in between. To improve cure rates and EFS of children with ALL in developing world induction remission is one of components to be used.

CHAPTER 3: STUDY JUSTIFICATION, QUESTION AND OBJECTIVES

3.1 Justification

Paediatric malignancies are on the rise in the developing world. This is more so in sub-Saharan Africa where on top of the common childhood illnesses caused by infective processes, oncologic cases are on the rise. This may be due to better surveillance or other factors but modalities for care need to improve in line with the developed world.[49, 50]

ALL is the second most common cancer in children under 15 year after Non Hodgkin Lymphoma in East Africa.[6, 64]

Categorization of childhood ALL is poorly done in the third world countries mainly due to financial constraints but this should change. To understand the presentation of these patients may assist to tailor treatment modalities to fit this setting. In the developed countries where treatment modalities have been tailored to the different prognostic indicators the EFS rates have improved markedly over time.[3, 10, 12]

Racial variance to the presentation has been alluded to, but not clearly delineated[52, 62], thus may contribute to treatment outcomes.

Challenges faced with chemotherapeutic agents sourcing and administration are common in this set up and studies in outcomes will assist in determining if they are worsening the situation.

It has been shown that induction failure in childhood ALL is one of the poor prognostic factors[7, 10, 12], thus monitoring the response might explain the poor survival rates[13, 63] seen in our set-up over the duration of therapy or throughout life.

This study looks at the response of children with ALL to induction chemotherapy which is a major factor in determining the likelihood of achieving good outcomes and cure.

3.2 Research Questions

1. What are the clinical and laboratory features of children who present with ALL in MTRH?
2. What is the clinical and laboratory response to induction chemotherapy of children with ALL in MTRH?

3.3 Objectives

3.3.1 Broad Objective

To determine the response of paediatric acute lymphoblastic leukemia to induction chemotherapy at MTRH

3.3.2 Specific Objectives

1. To describe the clinical and laboratory features of children who present with ALL at MTRH.
2. To describe the clinical and laboratory response to induction chemotherapy in children with ALL at MTRH.

CHAPTER 4: METHODOLOGY

4.1 Study design

This was a prospective study that involved use of quantitative techniques. This design was chosen so as to limit the amount of missing data and to standardise the data collected. The paediatric patients were followed for 6 weeks with specific monitoring of their laboratory parameters at day 42. The clinical characteristics' were recorded at diagnosis (baseline) and at the end of induction (42^{nd} day). All these patients were in the paediatric ward, oncology unit, where they were physical examined by the principal investigator.

4.2 Study site

This study was carried out at paediatric oncology unit in the paediatric wards of the Moi Teaching and Referral Hospital, Eldoret, Kenya. The Hospital is located in Eldoret town of the former Rift Valley province of Kenya, presently in Uasin Gishu County. It is located 300 Kilometres Northwest of the Kenya's capital city Nairobi. This is also the teaching hospital for the Moi University's School of Medicine, University of Eastern Africa, Baraton and the Kenya Medical Training College, Eldoret. It is the second largest national referral hospital in Kenya and serves patients from the western part of Kenya and the north and south Rift region. It has a catchment population of approximately 13 million people and does handle approximately 20,000 admissions annually. Approximately 30,000 children are seen at the paediatric outpatient department (OPD) per year. The main economic activity of the population is farming and business.

The paediatric oncology unit is situated in one of the general paediatric wards. It has a bed capacity of 16 with a team of dedicated health care providers.

The doctors in the unit were a medical officer (who has been in the unit for 3 years) and a paediatric oncologist.

4.3 Study population

The target population were all children admitted at the hospital during the study period between May 2012 and May 2013. The study population were all children admitted to the paediatric oncology unit in the paediatric wards with a diagnosis of ALL at Moi Teaching and Referral Hospital.

4.4 Sampling techniques

All children admitted to the oncology unit with the diagnosis of ALL and meeting the study's inclusion and exclusion criteria were consecutively recruited until the study duration ended.

4.5 Inclusion and exclusion criteria

The Inclusion criteria of the study were as follows:

1. All children newly diagnosed with ALL.
2. Consent given by parent or guardian (see appendix I).
3. Assent from the older child above 7 years of age (see appendix VIII).

The exclusion criteria of the study:

Child who had received chemotherapy previously.

4.6 Sample Size Determination

All the children meeting the criteria, presenting between May 2012 and May 2013 were recruited into the study.

4.7 Data Collection Methods

Clinicians at MTRH Sick Child Clinic (SCC) obtained medical history and physical examination of all children suspected of ALL. Blood was drawn for CBC and PBF investigations. Blood was drawn using the standard procedure in appendix VII with the PBF using the procedure as per appendix IV. The slide was read by 2 pathologists who fortunately didn't disagree on interpretation.

The principal investigator with the help of a research assistant (medical officer in oncology unit) recruited children at the stage of presumptive diagnosis in the paediatric wards and took a detailed history and physical examinations. This was recorded into the data collection form (see appendix II).

While in the ward, the diagnostic BMA (see appendix V) was done by clinicians and principal investigator using BMA procedure as shown in appendix V.

A diagnosis of ALL was made from the BMA. The BMA had to have more than 25% blasts for the diagnosis of ALL to be made. This was read by 2 pathologists independently. If they disagreed a third pathologist was called in to break the tie. No study participant was recruited without agreement between the pathologists on the diagnosis.

Upon confirmation of ALL, children were recruited into the study after obtaining assent and consent. The BMA test was done as per the MTRH paediatric oncology recommended sedation protocol (see appendix VI). Children were then started on treatment as per the MTRH protocol (see appendix III) in the oncology unit.

The diagnostic laboratory findings such as CBC, PBF and BMA was entered by the principal investigator and research assistant into data collection form (appendix II). The

CBC parameters used in this study included the absolute white blood cells count, the haemoglobin level, absolute lymphocyte count, and the absolute platelet count.

Upon completion of the induction phase of chemotherapy, the principal investigator or the research assistant evaluated the child both clinically and using the laboratory parameters. Clinical evaluation included checks for signs and symptoms while the laboratory parameters monitored the CBC, PBF and BMA. This was at done at day 42 as per the MTRH protocol. PBF was done at day 8 and at day 42 by the principal investigator.

The study outcomes was measured in terms of survival and death of the child. The cases which survived was further grouped as achieving remission status or those not achieving. Remission was assessed using the post-induction BMA.

Remission was achieved if the post-induction BMA had less than 5% blasts. Partial remission was noted when this BMA had blasts in the range of 5% to 25%, while no remission was when it had more than 25% blasts.

The PBF and BMA were read independently by two qualified pathologist and for purposes of quality control, the clinical aspects of the patient were included. A pathological diagnosis was then made upon the agreement of the two pathologists. The pathologist main focus was on the clinical remission i.e. the percentage of lymphoblasts in both the PBF and the BMA.

A prior induction course for clinicians and research assistant was done through study specific training at SCC and the paediatric wards.

4.8 Data analysis

Data was collected prospectively and entered into data collection form. Later, the data was transferred into a computer database and exported for analysis. All patient details was kept confidential and data were only available to the principal investigator and the research assistant. Copies of all results was put into the patient's file which was further used for treatment.

The guardians were informed about their children's condition when they wished so. Older children were also informed if they chose to know.

Data analysis was performed using STATA version 12 special edition. Continuous variables were summarized as median and the corresponding inter quartile range (IQR) while the categorical variables were summarized as frequencies and the corresponding percentages. The test for differences in continuous variables was conducted using Wilcoxon rank sum test. Comparison of the baseline and the six week or the eighth day lab results was done using Wilcoxon sign test. The test for association between the categorical variables was done using the Pearson's Chi Square test. The test for reduction in the symptoms was conducted using the McNemar's test. The p-values and the incidence proportions were reported. Survival analysis using the Kaplan-Meier curves was done.

4.9 Study Limitations

The main limitation in this study was lack of definitive B and T cell lymphoblastic leukemia induction treatment.

4.10 Ethical considerations

Approval to carry out the study was sought from IREC of MTRH and Moi University. Written informed consent was obtained from the parent or guardian of all children included in this study with children older than 7 years also giving their assent. All procedures were done with through explanation of possible benefits and potential risks.

Confidentiality was maintained throughout the study by consenting in semi-private consultation room, pass-wording the database and locking the filled data collection forms in lock and key data cabinets.

All participants received the same level of care as all other patients who were not participating in the study.

No inducements or incentives was used to convince patients, parents or guardians to participate in the study.

The results of this study were made available to all persons concerned with the care of children with ALL.

CHAPTER 5: RESULTS

5.1 Demographic characteristics

A total of 30 patients with ALL participated in this study. The demographic characteristics are shown in table 1 below. Male and female patients were not different from each other in age at enrolment, with median 8(IQR: 6-11) years, and 8.5(IQR: 5-13) years respectively (P=0.877).

Table 1: Demographic Characteristics

Characteristics	**Patients**
Age	**Years**
Range	1-14
Median	8
IQR	6-11
Less than 12 months	2(6.67%)
13 months - 10 years	20(66.67%)
Over 10 years	8(26.67%)
Sex	**n (%)**
Male	10(33%)
Female	20(67%)

5.2 Clinical presentation

The mean symptoms duration as at enrolment was 10 (Range: 1-36) weeks.

Other clinical presentations of the 30 participants are summarised as shown in table 2 below

Table 2: Clinical presentations of the participants (n=30)

Clinical Presentations	
Symptoms	**n (%)**
Weight loss	16(53%)
Night sweats	14(47%)
Bleeding tendencies	13(43%)
Bone pain	10(33%)
Signs	
Anaemia	28(93%)
Fever	25(83%)
Lymphadenopathy	18(60%)
Splenomegaly	11(37%)
Hepatomegaly	9(30%)

5.3 Laboratory findings

The median number of WBC count, number of platelets, and number of lymphocytes at enrollment are shown in Table 3. Only one participant, had CSF findings (CNS disease) at baseline.

Table 3: Baseline laboratory findings

Variable	**Median**	**IQR**
WBC count (X 10^9 /l)	45X 10^9 /l	13-101(X 109 /l)
Platelets count (X 10^9 /l)	25X 10^9 /l	14-51(X 109 /l)
Lymphocytes count (X 10^9 /l)	28X 10^9 /l	6-59(X 109 /l)
Hemoglobin level (g/dL)	5.8g/dl	4.6-8.7(g/dl)
PBF Lymphoblasts (percentage)	70%	40-80%
BMA Lymphoblasts (percentage)	90%	60-90%

Only one patient did not have any blasts on the PBF as shown in table 4 below.

Table 4: Laboratory parameters before induction

Parameters	**Patients n (Percentages)**
WBC count	**n (%)**
< 50X 10^9/l	16(53%)
>50X 10^9/l	14(47%)
Platelet count	**n (%)**
<150,000/ul	26(86%)
151,000/ul – 450,000/ul	2(7%)
>451,000/ul	2(7%)
Haemoglobin level	**n (%)**
Below 8g/dl	28(93%)
8g/dl and above	2(7%)
Lymphoblasts	
Blasts seen in PBF	29(96.7%); n=30
Blast cells seen (Range)	11% - 90%
Blasts seen in BMA	30(100%)
CNS Disease	**n (%)**
Present	1(3%)
Absent	29(97%)

5.4 Duration of Therapy

The median duration for the therapy was 42 days. The minimum duration was seven days and the maximum was 55 days as shown in table 5. The 4 participants who did not finish the minimum 42 days died.

Table 5: Duration of therapy for the participants

Duration of Therapy(Days)	**Patients**
Less than 42	4(13.3%)
42	22(73.3%)
More than 42	4(13.3%)

5.5 Clinical responses

The reduction in the severity of the symptoms and signs was assessed. McNemar's test to assess the reduction of the symptoms and signs was conducted and the results were as shown in Table 6 below.

There was a significant drop in the number of participants who had anemia after induction. Similarly, there were significant drop in the numbers that had fever, bone pain, hepatomegaly, lymphadenopathy, and those who lost weight. This is a clear indication that the induction worked in favor of most of them.

Table 6: Assessing the reduction in symptoms and signs after induction

			McNemar's test
Symptom/Sign	**Before treatment**	**After treatment**	**Exact P**
Anemia	28(93%)	6(20%)	<0.001
Fever	25(83%)	7(23%)	<0.001
Bleeding tendencies	13(43%)	6(20%)	0.119
Bone pain	10(33%)	-	0.002
Hepatomegaly	9(30%)	-	0.004
Night sweats	14(47%)	6(20%)	0.057
Lymphadenopathy	18(60%)	3(10%)	<0.001
Weight loss	16(53%)	4(13%)	0.008
Splenomegaly	11(37%)	4(13%)	0.066

5.6: Laboratory Response

The six week laboratory parameters were compared to the baseline to determine if there was any change. This was done using Wilcoxon sign rank test. The results are as shown in Table 7.

There was a significant drop in the number of WBC, and in the lymphocyte count at the sixth week. On the other hand, there was a significant increase in the platelets count, and in the level of hemoglobin.

The test for difference between the baseline PBF lymphoblasts and the 8th day lymphoblasts showed that the 8th day PBF lymphoblasts was significantly lower than that of the baseline, P<0.001.

Table 7: Test for change in the clinical findings

Variable	**Baseline** **Median (IQR)**	**Six weeks** **Median (IQR)**	**P**
WBC count (X 10^9 /l)	45(13-101)	5(3-9)	<0.001
Platelets count (X 10^9 /l)	25(14-51)	273(88-419)	<0.001
Lymphocytes count (X 10^9 /l)	28(6-59)	2(1-3)	0.002
Hemoglobin level (g/dL)	5.8(4.6-8.7)	10(9-12)	<0.001

5.7: Outcomes

The BMA lymphoblast at 6 weeks was measured. There were 23(77%) participants who had BMA lymphoblast below 5% (achieved remission) as shown in table 8.

Of the 29 participants who did not have CNS disease at baseline, 4(14%) died. The only participant who had CNS disease was alive by the close of the study.

Table 8: Bone marrow remission pattern at Day 42 (n=30)

Remission Pattern	**No. of patients**
Remission (<5% blasts)	23(77%)
Partial Remission (5-25% blasts)	2(7%)
No Remission (>25% blasts)	1(3%)
Patients who died	4(13%)

5.7.1. Association between Age at Presentation and Death

The number of participants who died was 4(13%). Of these were two each aged 1 year and two each aged 6 years. This leaves us with 26 surviving participants age>1 year by the close of the study.

All of those aged at most 1 year (<=1 year) and 2(10%) of those aged 1-10 years died. None among those aged >10 years died. There was significant association between mortality and age (Fisher's exact P=0.020), with younger children being more likely to die.

5.7.2. Association between Age and Remission status

Among those aged 1-10 years, 17(94%) had remission while among those aged >10 years 6(75%) had remission. The test for differences in these two incidence proportions showed no significant difference (Fisher's exact P=0.215).

5.7.3. Association between Initial Platelet count and Remission status

Three (87%) of those with platelets less than 150,000/ul had remission. All of those with platelets between 150,000/ul - 450,000/ul had remission and one of those with platelets more than 450,000 /ul had remission. The test for association between remission and platelets levels was not statistically significant.

All the deaths occurred among those who had platelets less than 150,000/ul.

5.7.4. Association between Initial Haemoglobin level and Remission status

Of those with hemoglobin less than 8g/dL, 17(89%) had remission while among those with hemoglobin at least 8g/dL, 6(75%) had remission. There was no evidence of any association between hemoglobin levels and remission (Fisher's Exact P=0.558).

5.7.5. Association between Initial Haemoglobin level and Death

Two of those having hemoglobin less than 8 g/dL died and two of those having hemoglobin at least 8 g/dL died. The test for association showed no evidence of association (Fisher's Exact P=0.563).

5.7.6. Survival

There were a total of 30 participants who were followed in the study. The total person days were 1160. Of the 30 participants, 4 died giving an incidence rate of 3.4 deaths per 100 person days.

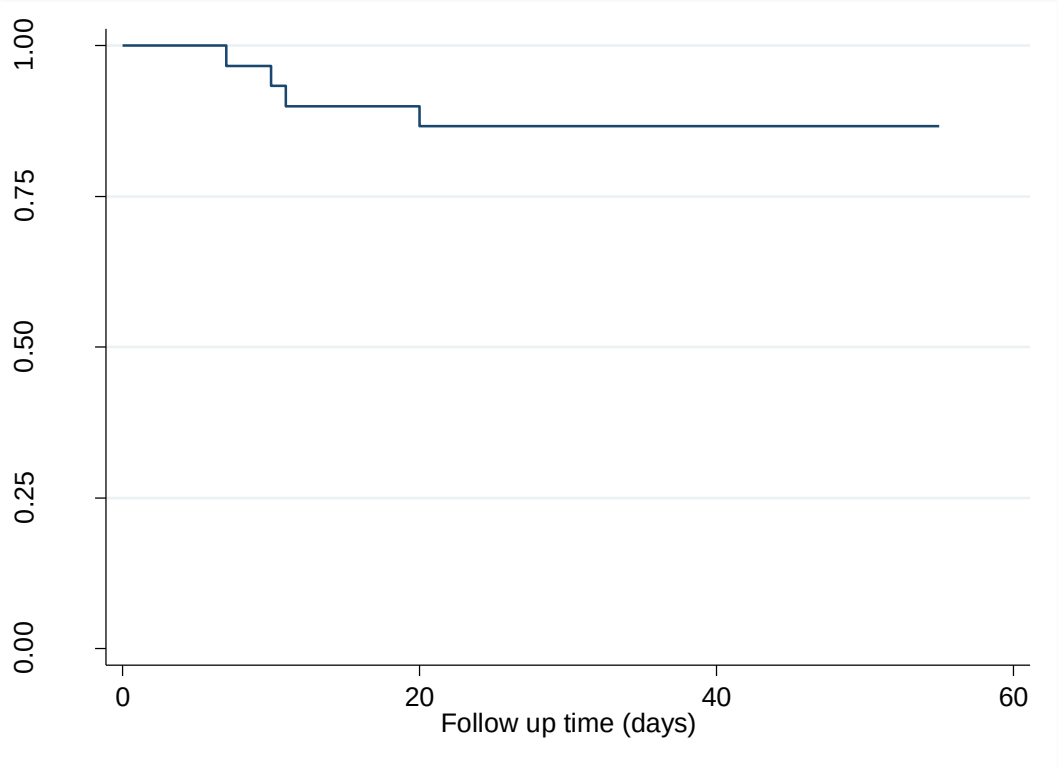


Figure 1: Survival function for time to death

CHAPTER 6: DISCUSSION

6.1 Demographic characteristics

This study sought to establish the clinical outcomes of induction among children seen at MTRH's paediatric oncology unit. There was a ratio of 1:2 (male to female) participants who underwent the induction phase. This is unlike a study by McNeil et al., in the United States of America that found that ALL is more prevalent among boys than girls among all age groups at any particular point in time[3]. Although this study was not a prevalence study, the number of boys admitted at the paediatric oncology unit, MTRH, with ALL were less than girls. This may be explained by the fact that the female gender is a good prognostic indicator compared to males due to the risk of testicular seeding of leukemic cells resulting in boys having a higher risk of death early on during presentation. Although in the developed countries with more intense therapy, the association between gender and outcome has become less or is no longer significant.[30, 55]

Another study done in India by Khalid S, et al, showed more males than females at a ratio of 3:1[57]. It included children up to 16 years in contrast with this study which had up to 14 years. This difference may be explained better if a prevalence study on paediatric ALL is done since both studies were hospital based.

Rana AZ et al did a study on Outcome of childhood Acute Lymphoblastic Leukemia after induction therapy – 3 year experience at a single paediatric oncology centre in Pakistan which showed more males to females at a ratio of 2:1[56]. The study was over 3 year period and retrospective in nature contrasting with this one which is prospective. Rana AZ et al were able to sample 38 patients with a mean age of 5.4 years, while this study had 30 with a median age of 8 years.

In a more recently published study done by Mushtaq N et al[58] with a larger sample size the male to female ratio was 1.8:1 showing that a disparity is evident though majority of the studies show that males are more affected than females. A reason for these discrepancy might be that in MTRH we are receiving children with good prognostic indicators, being female, while those with poor prognosis, males, don't reach the facility. Males can be affected with testicular leukemia. Testicular leukemia is classified as high risk with a lower EFS rate according to the clinical risk assessment of childhood ALL.[61]

The overall median age at enrolment was 8 years (IQR: 6-11) years and this is consistent with many studies that have found median ages to be around 6 years. Male and female patients were almost of the same ages at enrolment and they were not significantly different (P=0.877). This indicates that this condition affects all children but only more common between 2- 6 years as suggested by other studies.

The Khalid S et al study found a median age group of 6 with age range of 0.9 – 16 years from their sample size of 46 children[57]. In our study the age range was between 1 – 14 years from a sample size of 30 children. This could be the reason for the difference in the median age.

In this study two thirds of the children were in the age group of between 1 – 10 years which was the same as in a study by Mushtaq N et al which had majority (71%) at the same age group[58]. This is in spite the latter study having a larger sample size than this study.

According to the study in India by Khalid S et al, 58.7% of the patients were in the age group of one to nine years being the majority[57]. This being in agreement with the findings in this (MTRH) study.

This study found over a quarter of our participants were above 10 years old. This is less than what was found in an older study done by Macharia WN, where the proportions of children above the age of nine years constituted slightly above a third in developed countries and the Black African population[52]. Macharia WN, also found that the proportions of children below the age of two years was slightly above a quarter. This discrepancy may be due to the different age cut offs but more likely due to our study having a small sample size whereas Macharia WN did a review of published data.

In this study, majority of our participants were in the good prognostic age group of between 1 – 10 years which might be explained using the long mean duration of symptoms found in this study as late presentation, thus we might be losing out on the poor prognostic group who might not be reaching us.

6.2 Clinical presentation

Anaemia was the most common clinical presentation which is in keeping most of the other studies in the developing world. A case point is the Pakistan study done by Rana AZ et al which found pallor presentation in 84% of the presentations followed by fever at 73%[56]. Anaemia results from the excessive proliferation of lymphoid lineage of cells in the bone marrow with suppression of the erythroid lineage.[30] This is a sign of bone marrow failure.

In our study we found a very small proportion of the participants having CNS involvement at initial presentation. This in contrast with a review of published data carried out by Macharia WN which found proportions for CNS involvement in developed countries and developing countries were 5% and 13% respectively[52]. This can be explained by our study having a small sample size. In spite of the small sample

size an explanation for the few children with CNS disease could be that we are getting our patients late and those with CNS disease being a poor prognostic indicator[46] not making it to our hospital. This is alluded to by the long duration of symptoms in our study.

Khalid S et al found that the clinical characteristics that were noted at diagnosis included: lymphadenopathy, hepatomegaly, splenomegaly and CNS involvement of the patients in that order of occurence[57]. This is similar with this study which found, in order of occurrence: lymphadenopathy, splenomegaly, hepatomegaly and CNS involvement. Most patients with ALL tend to present with the above.

Khalid S et al found less CNS involvement as we did in this study, together with hepatomegaly and splenomegaly. Though the Khalid group relied more on recorded data since it was a retrospective study.

Mushtaq N et al in their Pakistan study found overt CNS symptoms in 11 (9.1%) cases[58]. This high CNS involvement compared to our study may be due to the larger sample size and the longer study period.

Approximately half of children may present with lymphadenopathy as reported by Margolin, JF et al[30] which is in tandem with this study in spite of the low sample size.

Fever (81%) and pallor (82.6%) were the most common presentation in a study done retrospectively from 1997 to 2006 by Mushtaq N et al[58]. This is in keeping with this study.

Noting that most common presentations are mainly due to bone marrow failure, this could be indicative of late presentation which may be alluded to by the mean symptoms

duration as at enrolment being 10 weeks(Range: 1-36). This may also explain the lymphadenopathy, which is one of the indications of extra medullary leukemic spread.

6.3 Laboratory Presentation

In this study we found a majority of our patients presenting with anaemia, this in line with most studies in the developing countries. Rana AZ, et al found the haemoglobin was in the range of 6–10 G/dl in most of the cases in the study done in Multan, Pakistan from December 2005 to December 2008[56]. This is still in keeping with bone marrow suppression stage deduced with the longer duration of symptoms.

In the study from India by Khalid et al, the mean initial blood counts at the time of presentation were as follows: haemoglobin: 7.8 g/dl; WBC count: 32.7 x 10^9 /L and platelets: 64 x 10^9 /L[57]. This when compared with findings in this study, is indicative still of still bone marrow suppression as our patients had low haemoglobin and platelet levels with high WBC count. This is in spite the Indian study having children up to 16 years of age.

Anaemia shown in our study indicates most of our children present already in bone marrow failure since they are either not producing enough red blood cells or are losing them. This is further supported by the long symptom duration at presentation to MTRH. Either the erythroid lineage and/or the myeloid lineage are affected and hence the bone marrow failure.

Majority of our patients presented with a WBC count of less than 50,000/ul, which is similar with the study done by Rana AZ et al, who had 73% of the participants having less than 50,000/ul WBC count[56].

Khalid et at reported 87% of the patients in that study had WBC count less than 50 x 10^9 /L[58]. In the study done by Mushtaq N et al, over 80% of the children had a WBC count of less than $50X10^9$ /L[59]. Both comparable with majority of the patients having WBC count of less than 50,000/ul as is the case in our study.

This presentation of the WBC counts (less than $50X10^9$/L) is a good prognostic indicator for our study participants. It may be explained using the long duration of symptoms to indicate late presentation of our children and hence a probability of not getting the children with the poor prognostic indicators at presentation.

6.4 Outcomes

Majority of our patients achieved complete remission. When compared with the study done by Rana AZ, et al where 74% of those patients achieved complete remission after 28 days of induction it illustrates how we compare favourably with them. This is with our induction chemotherapy lasting 14 days longer. The Rana et al study had 18% of its patients dying during course of induction therapy which is a slight increase compared to our study[56]. This slight difference may be linked to the intense 28 day induction compared to our longer induction period.

The retrospective study by Khalid et al showed that 97.8 % of the patients were in complete remission[57]. This study also had a shorter period of the induction chemotherapy by 14 days.

In the study done by Mushtaq N et al, remission (M1 marrow status) was attained in 97%. Death occurred in 7.4% of all the patients, during the induction phase (induction death rate of 8.5%) [58].

According to Silverman LB et al[53], failure of induction therapy is a relatively rare event occurring in fewer than 5% of children with ALL treated with current regimens. This shows how we compare with the developed world.

In all the above mentioned studies, internationally recognized protocols were used and all had L-asparaginase in their induction therapy. This agent was not used in our study. This might be the reason or a compounding factor for the not so robust response to induction chemotherapy in paediatric Acute Lymphoblastic Leukemia.

In this study we found an association between mortality and age at presentation. This is similar to a study done by Khalid S, et al in Pakistan which found one of the significant variables in terms of prognosis being age with a P value 0.04[57]. The similarity is present in spite of the study in Pakistan having a longer follow up period and a larger sample size.

In this study we found a low incidence rate of deaths per person days during the induction chemotherapy, this may not be comparable to other studies since most studies talk of cure rates for ALL after full treatment rather than only for the induction part. Also long term follow may be required which was not done in this study. Other studies also report survival rates after full treatment. An example of such a study is the one done in Pakistan by Mushtaq N et al, which found the event-free survival of 63% and overall survival was 65%, where the median follow-up was 28 months (IQR 8-43)[58].

The good outcome in this study may be explained with our patients presenting with good prognostic factors. This include majority being female, two thirds of our children in the age group of 1 – 10 years, majority had white blood cell count less than 50,000/ul and very few had CNS disease at presentation.

Although some inferences have been made, a note should be made of the small sample size in this study giving it a low power. This study is not powered enough to make conclusive inferences.

CHAPTER 7: CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

- Most patients had good prognostic factors which include female gender, age between 1-10 years, no CNS disease at presentation and a white blood cell count less than 50,000/ul.
- The main presentation is anaemia, fever and night sweats, alluding to a stage of bone marrow failure.
- Paediatric Acute Lymphoblastic Leukemia has a good response to induction chemotherapy. Most of the patients achieve complete remission.
- Patients had a long mean duration of symptoms further supporting the presentation of bone marrow failure.
- Few patients attained partial remission.
- Patients below the age of 1 year have poor outcomes.

7.2 Recommendations

- Further epidemiological studies on paediatric ALL to be done.
- Further studies that stratify patients are required.
- Long term studies on children with ALL to determine survival rates should be done.
- More aggressive treatment for those presenting below the age of 1 year is required.
- Stratification of patients using prognostic indicators should be done in order to modify treatment protocols

REFERENCES

1. Brunning, RD, Flandrin, G, Borowitz, M, et al. Precursor B lymphoblastic leukaemia/lymphoblastic lymphoma (Precursor B-cell acute lymphoblastic leukaemia). In: World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues, Jaffe, ES, Harris, NL, Stein, H, Vardiman, JW (Eds), IARC Press, Lyon, 2001. p.111.
2. Harris NL, Jaffe ES, Diebold J, Flandrin G, Muller-Hermelink HK, Vardiman J, Lister TA, Bloomfield CD. World Health Organization classification of neoplastic diseases of the hematopoietic and lymphoid tissues: report of the Clinical Advisory Committee meeting-Airlie House, Virginia, November 1997. J Clin Oncol. 1999; 17(12):3835-49.
3. McNeil DE, Cot? TR, Clegg L, Mauer A. SEER update of incidence and trends in pediatric malignancies: acute lymphoblastic leukemia. Med Pediatr Oncol. 2002; 39(6):554-7; discussion 552-3.
4. Ribera JM, Oriol A. Acute Lymphoblastic Leukemia in adolescents and young adults. Hamatol Oncol Clin North Am. Oct 2009; 23(5): 1033-45,vi.
5. Mwanda OW. Cancer in children younger than age 16 years in Kenya. East Africa Medical Journal. 1999 Jan; 76(1):3-9.
6. Macharia WM. Childhood cancers in a referral hospital in Kenya: a review. East African Medical Journal. 1996 Oct; 73(10):647-50.
7. Trigg ME, Sather HN, et al. Ten year survival of children with ALL: a report from the Children Oncology Group. Leu Lymphoma. 2008; 49(6):1142-54.
8. Pui CH, Relling MV, Downing JR. Acute lymphoblastic leukemia. N Engl J Med. 2004; 350: 1535-1548.
9. Nandakumar A, Anantha N, et al. Descriptive epidemiology of lymphoid and haemopoeitic malignancies in Bangalore, India. Int J Cancer. 1995; 63(1): 37-42

10. Oudot C, Auclerc MF, et al. Prognostic factors for leukemic induction failure in children with ALL and outcome after salvage therapy: the FRALLE 93 study. J Clin Oncol. 2008; 26(9):1496-503.
11. Boissel N, Auclerc MF, et al. Should adolescents with ALL be treated as old children or young adults? Comparison of French FRALLE 93 and LALA 94 trials. J Clin Oncol. 2003; 21(5): 774-80.
12. Cave' H, van der Werff ten Bosch J, et al. Clinical significance of minimal residual disease in childhood ALL. European organization for Research and Treatment of Cancer – Childhood Leukemia Cooperation Group. N Engl J Med. 1998; 339(9): 591-8.
13. Pulte D, Gondos A, Brenner H. Trends in 5- and 10-year survival after diagnosis with childhood hematologic malignancies in the United States, 1990 - 2004. J Natl Cancer Inst: 2008; 100(18): 1301–9.
14. Gaynon PS, Desai AA, et al. Early response to therapy and outcome in childhood acute lymphoblastic leukemia: a review. Cancer 1997; 80: 1717-26.
15. Gaynon PS, Bleyer WA, et al. Day 7 marrow response and outcome for children with acute lymphoblastic leukemia and unfavourable presenting features. Med Pediatr Oncol 1990; 18: 273-9.
16. Steinherz PG, Gaynon PS, et al. Cytoreduction and prognosis in acute lymphoblastic leukemia - the importance of early marrow response: report from the Children's Cancer Group. J Clin Oncol 1996; 14: 389-98.
17. Gurney JG, Severson RK, Davis S, Robison LL. Incidence of cancer in children in the United States. Sex-, race-, and 1-year age-specific rates by histologic type. Cancer. 1995; 75(8):2186-95.
18. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, Thun MJ. Cancer statistics, 2008. CA Cancer J Clin. 2008; 58(2):71-96.

19. Shah A, Coleman MP. Increasing incidence of childhood leukaemia: a controversy re-examined. Br J Cancer. 2007; 97(7):1009-12.

20. Gurney, JG, Bondy, ML. Epidemiologic research methods and childhood cancer. In: Principles and Practice of Pediatric Oncology (4th ed), Pizzo, PA, Poplack, DG (Eds), Lippincott-Raven, Philadelphia, 2001. p.13.

21. Buffler PA, Kwan ML, Reynolds P, Urayama KY. Environmental and genetic risk factors for childhood leukemia: appraising the evidence. Cancer Invest. 2005; 23(1):60-75.

22. Hjalgrim LL, Rostgaard K, Hjalgrim H, Westergaard T, Thomassen H, Forestier E, Gustafsson G, Kristinsson J, Melbye M, Schmiegelow K. Birth weight and risk for childhood leukemia in Denmark, Sweden, Norway, and Iceland. J Natl Cancer Inst. 2004; 96(20):1549-56.

23. Adelman AS, McLaughlin CC, Wu XC, Chen VW, Groves FD. Urbanization and incidence of acute lymphocytic leukemia among United States children aged 0-4. Br J Cancer. 2005; 92(11):2084-8.

24. Greaves M. Infection, immune responses and the etiology of childhood leukemia. Nat Rev Cancer. 2006; 6(3):193-203.

25. Kroll ME, Draper GJ, Stiller CA, Murphy MF. Childhood leukemia incidence in Britain, 1974-2000: time trends and possible relation to influenza epidemics. J Natl Cancer Inst. 2006; 98(6):417-20.

26. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR, Sultan C. Proposals for the classification of the acute leukemia. French-American-British (FAB) co-operative group. Br J Haematol. 1976; 33(4):451-8.

27. Miller DR, Leikin S, Albo V, Sather H, Hammond D. Prognostic importance of morphology (FAB classification) in childhood acute lymphoblastic leukemia (ALL). Br J Haematol. 1981; 48(2):199-206.

28. Lilleyman JS, Hann IM, Stevens RF, Richards SM, Eden OB, Chessells JM, Bailey CC. Cytomorphology of childhood lymphoblastic leukemia: a prospective study of 2000 patients. United Kingdom Medical Research Council's Working Party on Childhood Leukemia. Br J Haematol. 1992; 81(1):52-7.

29. Lilleyman JS, Hann IM, Stevens RF. The clinical significance of blast cell morphology in childhood lymphoblastic leukemia. Med Pediatr Oncol. 1986; 14(3):144-7.

30. Margolin, JF, Steuber, CP, Poplack, DG. Acute Lymphoblastic Leukemia. In: Principles and Practice of Pediatric Oncology (4th ed), Pizzo, PA, Poplack, DG (Eds), Lippincott-Raven, Philadelphia, 2001. p.489.

31. Harrison CJ. The detection and significance of chromosomal abnormalities in childhood acute lymphoblastic leukemia. Blood Rev. 2001; 15(1):49-59.

32. Harrison CJ, Moorman AV, Barber KE, Broadfield ZJ, Cheung KL, Harris RL, Jalali GR, Robinson HM, Strefford JC, Stewart A, Wright S, Griffiths M, Ross FM, Harewood L, Martineau M. Interphase molecular cytogenetic screening for chromosomal abnormalities of prognostic significance in childhood acute lymphoblastic leukemia: a UK Cancer Cytogenetics Group Study. Br J Haematol. 2005; 129(4):520-30.

33. Heerema NA, Sather HN, Sensel MG, Zhang T, Hutchinson RJ, Nachman JB, Lange BJ, Steinherz PG, Bostrom BC, Reaman GH, Gaynon PS, Uckun FM. Prognostic impact of trisomies of chromosomes 10, 17, and 5 among children with acute lymphoblastic leukemia and high hyperdiploidy (>50 chromosomes). J Clin Oncol. 2000; 18(9):1876-87.

34. Harris MB, Shuster JJ, Carroll A, Look AT, Borowitz MJ, Crist WM, Nitschke R, Pullen J, Steuber CP, Land VJ. Trisomy of leukemic cell chromosomes 4 and 10 identifies children with B-progenitor cell acute lymphoblastic leukemia with a very low risk of treatment failure: a Pediatric Oncology Group study. Blood. 1992; 79(12):3316-24.
35. Ross ME, Zhou X, Song G, Shurtleff SA, Girtman K, Williams WK, Liu HC, Mahfouz R, Raimondi SC, Lenny N, Patel A, Downing JR. Classification of pediatric acute lymphoblastic leukemia by gene expression profiling. Blood. 2003; 102(8):2951-9.
36. Mosquera-Caro, M, Helman, P, Veroff, R, et al. Identification, validation, and cloning of a novel gene (OPAL1) and associated genes highly predictive of outcome in pediatric acute lymphoblastic leukemia using gene expression profiling. Blood 2003; 102:4a.
37. Fine BM, Stanulla M, Schrappe M, Ho M, Viehmann S, Harbott J, Boxer LM. Gene expression patterns associated with recurrent chromosomal translocations in acute lymphoblastic leukemia. Blood. 2004; 103(3):1043-9.
38. van Delft FW, Bellotti T, Luo Z, Jones LK, Patel N, Yiannikouris O, Hill AS, Hubank M, Kempski H, Fletcher D, Chaplin T, Foot N, Young BD, Hann IM, Gammerman A, Saha V. Prospective gene expression analysis accurately subtypes acute leukaemia in children and establishes a commonality between hyperdiploidy and t(12;21) in acute lymphoblastic leukaemia. Br J Haematol. 2005; 130(1):26-35.
39. Holleman A, den Boer ML, de Menezes RX, Cheok MH, Cheng C, Kazemier KM, Janka-Schaub GE, G?bel U, Graubner UB, Evans WE, Pieters R. The expression of 70 apoptosis genes in relation to lineage, genetic subtype, cellular drug resistance, and outcome in childhood acute lymphoblastic leukemia. Blood. 2006; 107(2):769-76.

40. Raimondi SC, Zhou Y, Mathew S, Shurtleff SA, Sandlund JT, Rivera GK, Behm FG, Pui CH. Reassessment of the prognostic significance of hypodiploidy in pediatric patients with acute lymphoblastic leukemia. Cancer. 2003; 98(12):2715-22.
41. Harrison CJ, Moorman AV, Broadfield ZJ, Cheung KL, et al. Three distinct subgroups of hypodiploidy in acute lymphoblastic leukaemia. Br J Haematol. 2004; 125(5):552-9.
42. Shurtleff SA, Buijs A, Behm FG, Rubnitz JE, et al. TEL/AML1 fusion resulting from a cryptic t(12;21) is the most common genetic lesion in pediatric ALL and defines a subgroup of patients with an excellent prognosis. Leukemia. 1995; 9(12):1985-9.
43. Loh ML, Goldwasser MA, Silverman LB, et al. Prospective analysis of TEL/AML1-positive patients treated on Dana-Farber Cancer Institute Consortium Protocol 95-01. Blood. 2006; 107(11):4508-13.
44. Schneider NR, Carroll AJ, Shuster JJ, et al. New recurring cytogenetic abnormalities and association of blast cell karyotypes with prognosis in childhood T-cell acute lymphoblastic leukemia: a pediatric oncology group report of 343 cases. Blood. 2000; 96(7):2543-9.
45. Sinigaglia R, Gigante C, Bisinella G, et al. Musculoskeletal manifestations in pediatric acute leukemia. J Pediatr Orthop. 2008; 28(1):20-8.
46. Bleyer WA. Central nervous system leukemia. Pediatr Clin North Am. 1988; 35(4):789-814.
47. Ingram LC, Fairclough DL, Furman WL, Sandlund JT, Kun LE, Rivera GK, Pui CH. Cranial nerve palsy in childhood acute lymphoblastic leukemia and non-Hodgkin's lymphoma. Cancer. 1991; 67(9):2262-8.
48. K?rholz D, Bruder M, Engelbrecht V, R?ther W, G?bel U. Aseptic osteonecrosis in children with acute lymphoblastic leukemia. Pediatr Hematol Oncol. 1998; 15(4):307-15.

49. Metzger ML, Howard SC, Fu LC, et al. Outcome of childhood acute lymphoblastic leukemia in resource-poor countries. Lancet 2003; 362(9385): 706-8.

50. Howard SC, Pedrosa M, Lins M, et al. Establishment of a paediatric oncology program and outcomes of childhood acute lymphoblastic leukemia in a resource-poor area. JAMA 2004; 291(20): 2471-5.

51. Marwaha RK, Kulkarni KP, Bansal D, Trehan A. Pattern of mortality in childhood acute lymphoblastic leukemia: experience from a single center in northern India. J Pediatr Hematol Oncol. 2010 Jul; 32(5):366-9.

52. Macharia WM. Comparison of prognostic determinants in childhood acute lymphoblastic leukemia in Negroid and Caucasian populations. East African Medical Journal 1996 Oct;73(10): 638-42

53. Silverman LB, Gelber RD, et al. Induction failure in ALL of childhood. Cancer. 1999; 85(6):1395-404.

54. Klimza MJ, Sońta-Jakimczyk DJ. Prognostic value of the initial response to corticosteroids for children with acute lymphoblastic leukemia. Wiad Lek. 2005; 58(11-12): 622-5.

55. Hann I, Vora A, Harrison G, Harrison C, Eden O, Hill F, Gibson B, Richards S, UK Medical Research Council's Working Party on Childhood Leukaemia. Determinants of outcome after intensified therapy of childhood lymphoblastic leukemia: results from Medical Research Council United Kingdom ALL XI protocol. Br J Haematol. 2001; 113(1): 103-14.

56. Rana AZ, Rabbani MW, Sheikh MA and Khan AA. Outcome of Childhood Acute Lymphoblastic Luekemia after induction chemotherapy – 3 years' experience at a single paediatric oncology centre. J. Ayub Med. Coll Abbottabad 2009; 21(4): 150-153.

57. Khalid S, Moiz B, Adil SN and Khurshid M. Retrospective review of pediatric patients with Acute Lymphoblastic Leukemia: A single centre experience. Indian Journal of Pathology and Microbiology. 2010; 53(4): 704-710.

58. Mushtaq N, Fadoo Z and Naqui A. Childhood Acute Lymphoblastic Leukemia: experience from a single centre tertiary care facility of Pakistan. The Journal of Pakistan Medical Association, JPMA. 2013 Nov; 63(11): 1399-404.

59. Lilleyman JS. Clinical important of speed of response to therapy in childhood Lymphoblastic leukemia. Leuk. Lymphoma. 1998; 31:501-6.

60. Schrappe M, et al Improved outcome of childhood acute lymphoblastic leukemia.. Results of trial ALL-BFM 90. German-Austrian-Swiss ALL-BFM study Group. Blood 2000; 95:3310-22.

61. Schultz KR, Bowman WP, Aledo A, et al. Improve early event-free survival with imatinib in Philadelphia chromosome-positive acute lymphoblastic leukemia: a children's oncology group study. J Clin Oncol 2009; 22:5175.

62. Macdougall LG, Jankowitz P, Cohn R, Bernstein R. Acute childhood leukemia in Johannesburg. Ethnic differences in incidence, cell type and survival. Am J pediatr Hematol Oncol. Spring 1986; 8(1):43-51.

63. Togo B, Traore F, Diakite AA, Diallo S, Traore B, et al. Childhood acute lymphoblastic Leukimia: 12 cases in Mali Med Trop (mars). Dec 2011:71(6):629-31.

64. American Cancer Society. Global cancer facts and figures 2nd edition. Atlanta: American Cancer Society; 2011.

APPENDICES

APPENDIX I

Consent form

I, Dr. Ahoya Phinehas Ademi resident in the Department of Child Health and Paediatrics, Moi University, am carrying out a study on Response to Induction Chemotherapy in Paediatric Acute Lymphoblastic Leukemia at Moi Teaching and Referral Hospital, Eldoret.

The study involves follow up of a child during the period of getting the initial part of treatment lasting six weeks and documenting all findings during this period. This will involve documenting all tests done and physical findings during the six weeks. No additional investigations will be ordered for the purposes of this study.

There will be no direct benefits of participating in this study. Study subjects will be accorded same quality of management as non-study subjects.

All information obtained in this study will be treated with utmost confidentiality and shall not be divulged to any unauthorized person.

Participation in this study is voluntary, there is freedom to refuse to take part or withdraw at any time.

Sign or make a mark if you agree to take part in the study

Parent/Guardian: Investigator: Date:

…….. …… …….

APPENDIX II

DATA COLLECTION FORM

DEMOGRAPHICS

Study Number: .....

Medical Record Number: ……

Date of Birth: …….. Sex: Male ☐ Female ☐

CLINICAL PRESENTATION

Duration of symptoms.....

Bleeding tendencies ☐ Night sweats ☐ Weight loss ☐

Fever ☐ Hepatomegaly ☐ Splenomegaly ☐

Anaemia ☐ Bone pain ☐

Lymphadenopathy ☐ Sites of lymphadenopathy.....

CNS manifestations.....

INITIAL LABORATORY FINDINGS

WBC count.....X 10^9/l Lymphocyte count.....X 10^9/l

Platelet count.....X 10^9/l Haemoglobin level.....g/dl

PBF lymphoblasts..... %

BMA lymphoblasts ..... %

CSF findings.....CNS disease: Absent ☐

Present ☐ Number of lymphoblasts.....

Date of Starting Induction Therapy: …….....

Date of Induction Therapy Completion: .....

CLINICAL RESPONSE

Bleeding tendencies ☐ Night sweats ☐ Weight loss ☐

Fever ☐ Hepatomegaly ☐ Splenomegaly ☐

Anaemia ☐ Bone pain ☐

Lymphadenopathy ☐ Sites of lymphadenopathy.....

CNS manifestations.....

LABORATORY RESPONSE

WBC count.....X 10^9/l Lymphocyte count.....X10^9/l

Platelet count.....X 10^9/l Haemoglobin level.....g/dl

PBF lymphoblasts after 1week (day 8) of chemotherapy.....%

PBF lymphoblasts after 2 weeks (day 15) of chemotherapy..... %

BMA lymphoblasts at 6 weeks (day 42)..... %

PBF lymphoblasts at 6 weeks (day 42)..... %

CSF findings.....CNS disease.....Absent ☐

Present ☐ Number of

lymphoblasts..... Survival ....Remission status.....Absent ☐

Present ☐

Death..... ☐ Number of days they got chemotherapy.....

Reasons for not completing chemotherapy other than death

.....

.....

APPENDIX III

MTRH ALL INDUCTION PROTOCOL

ALL MANAGEMENT

- ✓ Do CXR, diagnostic bone marrow. Thrombocytopenia is no contra indication!
- ✓ Stabilize as indicated with packed cells till Hb >10 mg/dl unless WBC > 50.000, then aim at Hb 8 mg/dl Platelets transfusion if platelets < 10 x 10^9 or if bleeding. Treat infections
- ✓ Do baseline investigations as HIV, creatinine, electrolytes, uric acid, ALT, BS for MP's, stool for microscopy
- ✓ Do diagnostic lumbar puncture once platelets >50x10^9, give IT methotrexate and hydrocortisone. If necessary do LP after platelet transfusion. Use sedation and good LP technique to prevent leukemic spread.
- ✓ Start PO Allopurinol 10mg/kg in TID ideally 24 hours before start of prednisone / chemotherapy
- ✓ Start PO Prednisone 60mg/m^2 in TID (lower with WBC >50.000 x 10^6: Start 10mg/m^2, increase daily with 10 mg/m^2 till the dose of 60 mg/m^2 is reached at day 6.
- ✓ Hydrate 24 hours before and after cytotoxics, 3000 ml/m^2 with bicarbonate 40 mEq/l as long as tumour load is high. Aim is urine pH of 7-8 at start of prednisone or chemotherapy, adjust bicarbonate dose if necessary!

XXXXX = do not give

INDUCTION	**DATE** **DOSE**	**Wk 0**	**Wk 1**	**Wk 2**	**Wk 3**	**Wk 4**	**Wk 5**	**Wk 6**	
IV Vincristine 2mg/m^2 (max 2.5 mg)		XXX						XXX	BONE MARROW
IV Adriamycin 25 mg/m^2 (infusion one hr.)		XXX	XXX			XXX	XXX	XXX	
IM L-Asparaginase 6000U/m^2 (3 doses)		XXX	XXX	XXX	XXX	↓	↓ ↓	XXX	
IT Methotrexate / Hydrocortisone			XXX		XXX		XXX		
In CNS disease ** 3 drugs weekly IT Methotrexate / Hydrocortisone and Cytosar		XXX	*	*	*	*	*	*	
PO Prednisone 60mg/m^2 in TID week 1,2 and 4,5					XXX			XXX	
Signature									
Name									

Do weekly haemogram with peripheral blood film. Do bone marrow to check for remission in week 6.

Low WBC count is no contra indication to continue with IV Vincristine, l-asparaginase and prednisone!!

If no CNS disease give IT alternate weeks as per schedule

In CNS disease: combine IT Cytosar, Hydrocortisone and Methotrexate and give weekly till end of induction or for minimum of four weeks.

Intra-thecal drug dosages (Diluents should not contain preservatives!)

DRUG\ AGE	< 1 year	1-2 years	2-3 years	3-4 years	≥5 years
Methotrexate	6 mg	8mg	10 mg	12 mg	12 mg
Cytosar	15 mg	20 mg	25 mg	30 mg	40 mg
Hydrocortisone	6 mg	8mg	10 mg	12 mg	12 g

If no circulating blasts, IT can be given if platelets are >20 $x10^9$/L

Adapted from the MTRH paediatric oncology manual September 2010.

APPENDIX IV

PERIPHERAL BLOOD FILM PREPARATION

Slide preparations —peripheral blood smears will be prepared using either the wedge technique or the particle crush technique, manually.

A clean slide, free from dust, dirt, grease, and fingerprints, is used.

The slides will be alcohol-cleaned.

Smears will be made from one small drop of blood which has not been allowed to clot, and which has been completely mixed, from an anti-coagulated blood sample.

Procedure:

The Wedge technique —the drop of blood is placed at the end of one slide and spread evenly down the length using a second glass slide. The second slide should be angled at 30 degrees to the base slide; the drop should be pushed using a rapid, even motion.

Particle crush technique — a small drop of blood is placed at the end of a glass slide. A second glass slide is held parallel and directly over the drop of the aspirate. The second slide is then pressed against the drop of aspirate and pulled across the full length of the first slide.

Staining procedure:

- The slides will be stained with leishman stain.
- The slide is flooded with the stain (7-10 drops) for 5 minutes.
- Buffer of distilled water is added (10-15 drops) for 20-30 minutes.
- Slide is washed and cleaned around the periphery (back) with the buffer.
- Then air dried.

APPENDIX V

BONE MARROW ASPIRATE PROCEDURE

Materials:

- Sterile gloves
- Povidone-iodine antiseptic solution for cleansing the chosen site, along with the necessary drapes to maintain sterility at the site
- A 1 or 2 percent lidocaine solution for local anaesthesia, along with a sterile syringe, a 23-gauge and a 21-gauge needle
- A number 11 scalpel blade for making the skin incision prior to inserting the aspiration and biopsy needles
- Sufficient quantities of sterile gauze and bandages to clean the biopsy site and to apply local pressure to insure haemostasis when the procedure has been completed
- A large selection of needles used for aspiration is available. A needle with a stylet that can be fixed in place initially and removed later is preferred. It is important to use a sharp needle, as well as one long enough to penetrate through the subcutaneous tissues and into the marrow cavity.
- Jamshidi biopsy needle with stylet and a device (obturator) for removing the biopsy core from the needle without damage to the specimen.

Choice of aspiration site:

- The posterior superior iliac crest and spine is the favoured site of examination in the child and in most infants. This site also provides the least discomfort to the patient compared with other sites.
- The anterior iliac crest may be used for bone marrow aspiration and biopsy when access to the posterior iliac crest is limited (for example, the patient is unable to be moved for

proper access to the chosen aspiration site, morbid obesity, skin diseases, or previous radiation). An initial attempt to sample the posterior iliac bone may be worthwhile, even in neonates.

- Obtaining bone marrow from a site which has been previously irradiated is likely to yield suboptimal results, especially in terms of overall cellularity. Another site should be chosen if at all possible.
- Sternal aspiration should not be undertaken in young children due to the risk of perforating the sternal plate. For the same reasons, marrow biopsy at sternal sites is contraindicated.

Premedication- as per the MTRH paediatric oncology protocol on sedation (see appendix VI) ketamine will be used as it is recommended for sedation during BMA.

Use of an assistant —a trained assistant from the haematology laboratory in MTRH will be used to help prepare the slides and specimens. Another assistant will be available to monitor the patient vital signs during the procedure.

TECHNIQUE

Posterior iliac crest:

- Premedication's (anti-anxiolytics or pain medications) be administered.
- The patient be placed in either a prone or lateral decubitus position. In heavier patients, a lateral decubitus position with the knees pulled closer to the chest, or a prone position accompanied by pressure over the bone, may help to identify the landmarks by reducing the depth of the fat pad overlying the iliac crest.
- Examine the potential site for evidence of infection; palpate the posterior iliac crest and posterior superior iliac spine and locate these landmarks. The anterior superior iliac spine should also be palpated and located, as the needle will be pointed in this direction once the bone has been entered.

- Maintain a steady dialogue with the patient, explaining each step, alerting the patient to potential discomfort, with reassurance as needed. This is in a situation whereby no sedation is used.
- In the absence of local skin problems (for example, infection, indurations, ulceration), the usual site for aspiration and biopsy is approximately three finger-widths from the midline and two finger-widths inferior to the iliac crest. Mark the chosen area by making an indentation in the skin with a coin, fingernail, or the end of a ballpoint pen with the writing tip retracted.
- Using sterile technique, protective clothing and gloves (and eye wear if necessary), the bone marrow tray should be first opened and organized for easy access to needed items. Needles, stylets, and plastic (not glass) syringes should be checked to ensure that they are intact and functioning properly.
- Cleanse the chosen area with povidone-iodine solution and drape a sterile field. Prepare the instruments.
- Anesthetize the skin and subcutaneous tissues with a 1 to 2 percent lidocaine solution using a 23-gauge needle; then anesthetize the periosteum by repeatedly injecting small amounts of lidocaine solution at different points on the surface of the bone with a 21-gauge needle. Anesthetize a dime-sized area of the periosteum surrounding the targeted location.
- Once local anaesthesia has been achieved, make a small (3 mm) skin incision with a scalpel blade at the site of insertion of the aspiration needle, in order to facilitate its entry and promote organized healing of the wound.
- Hold the bone marrow needle (with stylet in place) perpendicular to the skin at the previously marked point, and gently advance it to the periosteum. In order to be sure that the needle is entering correctly, the second and third fingers on the hand not being

used to insert the needle should be placed on the iliac crest or spine and the needle inserted between them. When the needle has been advanced to the periosteum, it should be pointed in the direction of the anterior superior iliac spine.

- Use a steady twisting back and forth motion. Do not twist more than 180 degrees to penetrate the periosteum and the cortical bone; a "give" is felt when the needle enters the marrow cavity. At the point of entry, the patient may express complaints of a deep-seated pain. It is important to alert the patient to this possibility ahead of time. Continue to advance the needle slightly to ensure that it is anchored into the bone.
- Remove the stylet, attach a 2 millilitre syringe to the aspiration needle, and again advise the patient that the aspiration may cause a brief period of pain.
- Aspirate 0.2 to 0.5 mL of marrow contents and remove the syringe. In general it is prudent to avoid aspirating more than 0.5 mL per syringe, as greater amounts may be prone to contamination with peripheral blood or to clotting.
- The non-anticoagulated specimen should be handed to the assistant, who will assess the quality of the sample (for instance, determine the presence or absence of grossly visible bone spicules) and prepare the various smears. The patient should be made aware of the need for multiple specimens at the outset, since each separate aspiration may be painful, despite fully adequate local anaesthesia.
- If aspiration attempts are not successful, reinsert the stylet (the needle may be rotated) and advance the needle a short distance; repeat attempts at aspiration with the syringe and suction. If multiple aspiration attempts are unsuccessful, an alternate site (for example, the other posterior iliac crest) may be approached with the same sterile strategy after the bone marrow biopsy has been obtained.

- Once it has been determined that the aspirate is satisfactory, reinsert the stylet, and remove the needle (with stylet in place) by using a similar twisting motion, and apply pressure to the site with small gauze square until the bleeding stops.
- If a biopsy is necessary, prepare the Jamshidi needle and advance it into the cortical bone, using the same incision but a slightly different site, with a steady twisting movement until it is firmly lodged. This may require a greater amount of pressure than was used for the aspiration. Remove the stylet and with a rotating motion advance the needle another 15 to 20 mm.
- Redirect the needle tip and rotate it 360 degrees in both directions to separate the biopsy specimen from the surrounding marrow tissue. Following this step, the needle should be advanced a very short distance prior to removal. This step may prevent the specimen from being pulled out of the needle at the biopsy site.
- Remove the needle with a slight twisting motion, place a sterile dressing over the site, and apply pressure for several minutes until the bleeding stops. Once haemostasis is achieved, a bandage should be applied, and the patient should be instructed to lie supine for 10 or more minutes. Pressure dressings may be required in thrombocytopenic patients.
- Once the biopsy needle has been removed, the specimen may be extracted from the needle by inserting the obturator (or stylet) through the distal (cutting) end of the needle. The bone marrow biopsy can then be placed on a slide, where imprints ("touch prints") are made before the core specimen is further processed for cytologic investigations. This step is especially useful in situations where a bone marrow aspirate could not be obtained.
- Examine the biopsy specimen. If the specimen consists mostly of homogeneous, white material (cortical bone) or glistening tissue (cartilage), it may be necessary to attempt a

second biopsy for a more satisfactory specimen. This should be done with a new biopsy needle, as the original needle may have been damaged by the process of inserting the obturator or stylet through the distal end of the biopsy needle.

- Prior to leaving the patient, the bone marrow aspiration/biopsy site(s) should be evaluated to assess for prolonged bleeding. This is minimized by applying a pressure dressing over the site(s), with the patient remaining recumbent, for at least 10 minutes.

Anterior iliac crest — Samples are taken from a site 2.5 to 5 cm posterior the anterior superior iliac spine and beneath the palpable lip of the iliac crest.

- The patient should be relaxed, comfortable, and in the supine position.
- Once a satisfactory site has been identified, the remainder of the procedure is identical to that for the posterior iliac crest (see above).

PREPARATION OF SAMPLES — Slides of the aspirated marrow will be prepared at the bedside. Depending on the clinical scenario, up to 9 bone marrow direct smears may be prepared.

When performed at the bedside, slides should be prepared rapidly to avoid clotting. Adequacy of the specimen is determined by the presence of "spicules," which appear as fatty droplets, granules, or small chunks of bone, which allow assessment of marrow cellularity. Spicules tend to concentrate at the feathered edge when a smear of the bone marrow is made.

Materials:

- Glass slides and coverslips. Both should be clean and free of dirt, grease, oil, or fingerprints

Procedure — Place the glass slides in a convenient location before the procedure to allow quick and easy access. Place approximately 0.5 mL of marrow aspirate on one

glass slide; if only one or two aspirate samples are to be prepared, the remainder may be added to an EDTA-anticoagulated sample collection tube, mixing well. This should be done immediately to prevent clotting of the specimen. Preparations are then made using one or more of the following two techniques:

i. Wedge technique — A few "particles" (spicules) are placed at the end of one slide and spread evenly down the length using a second glass slide. The second slide should be angled at 30 degrees to the base slide; the drop should be pushed using a rapid, even motion, ending in a particle (spicule)-rich feathered edge.

ii. Particle crush technique — a small drop of aspirated marrow is placed at the end of a glass slide. A second glass slide is held parallel and directly over the drop of the aspirate. The second slide is then pressed against the drop of aspirate and pulled across the full length of the first slide to crush open and spread the marrow particles.

The slides will then be taken to the MTRH laboratory for staining with leishman stain (see in appendix IV under staining procedure).

POST-PROCEDURE INSTRUCTIONS — following the procedure, the patient should lie in a supine position, so as to apply body weight to the biopsy site, for at least 10 to 15 minutes. The site should then be inspected to ensure that there is no further bleeding. The patient should be advised that the procedure site may be slightly tender for several days. The following additional routine instructions should be given:

- For pain control, a non opiod analgesia for 24 to 48-hour period for example: Paracetamol given orally at 15mg/kg/dose every 6-8 hours, or Ibuprofen orally 10mg/kg/dose every 8 hours.
- The patient be directed to contact the physician or clinic if swelling, marked tenderness, increased pain, and/or further bleeding is observed.

- The patient should avoid overexertion (for example, heavy activity or exercise) for at least 24 hours, to avoid potential pain or bleeding at the site of the procedure. The area of the aspiration/biopsy should be kept dry during this time to minimize the chance of infection or bleeding.

  If the patient continues to bleed from the aspirate/biopsy site after an initial observation period of 10 to 15 minutes, it may be prudent to reapply pressure to the site and have the patient lie supine for at least one hour. If bleeding continues after this additional time of observation, it may be necessary to transfuse platelets if the patient is severely thrombocytopenic or if platelet function is suspected to be compromised.

COMPLICATIONS

Risk factors for an adverse event included diagnosis of a myeloproliferative disorder, treatment with aspirin or warfarin, obesity, or disseminated intravascular coagulation. In general, however, when complications do occur, they tend to be minor, mainly consisting of bleeding at the biopsy/aspiration site or infection.

Bleeding — Haemorrhage from bone marrow aspiration can occur at any site, is more likely in the individual with thrombocytopenia and/or abnormal platelet function, and is associated most commonly with the myeloproliferative disorders. Of interest, the risk of haemorrhage has not been found to be associated with operator experience.

In most cases, bleeding is controlled by manual application of pressure to the site. Pressure dressings should be applied to the site following the procedure in patients with thrombocytopenia. If bleeding continues, platelet transfusions may have to be given if the patient is severely thrombocytopenic, or if platelet function is compromised.

There have been rare reports of retroperitoneal haemorrhage as well as gluteal artery laceration.

Infection — Infections are usually minor, requiring only topical medications. More serious infections may occur in immunocompromised patients. There is a potential risk of contracting infections from a patient, and some recommend double-gloving. However, universal precautions should be applied in all cases, and the operator should always take care to avoid needle penetration of the skin.

Tumour seeding — There have been rare case reports of tumour seeding from the bone marrow into the needle track (for example, into muscle, subcutaneous tissue, skin) following bone marrow biopsy, in patients with small cell lung carcinoma, multiple myeloma, and lymphoma.

Needle breakage — rarely, a bone marrow needle may break. If this occurs, an attempt to extract the distal segment with a haemostat should be made. If this manoeuvre is unsuccessful, a surgeon should be consulted.

Other — Seldom, patients may experience persistent discomfort at the site of biopsy. Exceedingly rare complications have included transient neuropathy with gluteal compartment syndrome secondary to post-biopsy bleeding, fracture due to underlying osteoporosis, and osteomyelitis. There may be abnormal radiologic studies of the pelvis post-biopsy, including lytic lesions surrounded by a sclerotic border, exostoses, or increased bone-seeking isotope uptake.

Adapted from UpToDate Version 18.3: Topic last updated August 13, 2010.

APPENDIX VI

PEDIATRIC SEDATION PROTOCOL FOR CHILDREN ≥ 3 YEARS

AIMS OF THE PROTOCOL

1. To facilitate the performance of minor procedures (e.g. intrathecal drug administration, bone marrow aspirates/biopsies and lymph node biopsies) in the ward procedure room.

2. To minimize the potential risks of cardiorespiratory complications of sedo-analgesia.

**For children under the age of 3 years consult the anaesthesiologist*.

PREPARATION FOR SEDO-ANALGESIA

1) Equipment.

Suction machine with catheter & tubings.

Bag Valve Mask – Ambu bag.

Oxygen source.

Face masks – Various sizes.

IV canulae – G 24, 22.

Needles – G 21, 23.

Syringes – 2, 3, & 10 cc.

Oral airway-various sizes

2) Drugs

Atropine 1 mg/mL.

Adrenaline 1mg/mL.

Ketamine 50 mg/mL.

Midazolam (Dormicum) 1mg/mL.

Lidocaine 2%

3) IV Fluids

Normal Saline

Ringer's lactate

4) Personnel

The Doctor performing the procedure

Doctor/Qualified nurse/clinical officer responsible for monitoring cardiorespiratory function during procedure

DILUTION OF DRUGS

1. Ketamine – Draw 1 cc (50 mg) in a 10 cc syringe and add 9 cc of Normal Saline to make 10 cc. The final concentration will be 5mg/mL.

2. Midazolam (Dormicum) – Draw 5cc (5 mg) in a 10 cc syringe and add 5 cc of Normal Saline to make 10 cc. The final concentration will be 0.5 mg/mL.

3. Atropine – Draw 1 cc (1mg) in a 10 cc syringe and add 9 cc of Normal saline to make 10 cc. The final concentration will be 0.1 mg/mL.

4. Adrenaline (1:1000) – Draw 1 cc (1mg) in a 10 cc syringe and add 9 cc of Normal saline to make 10 cc. The final concentration will be 0.1 mg/mL.

5. Lidocaine – Draw 5cc (100 mg) in a 10 cc syringe and add 5 cc Normal saline to make 10 c.c. The final concentration will be 10 mg/mL.

PROCEDURE FOR SEDATION

(i) Confirm that the patient has fasted for at least 6 hours.

(ii) Get patient's ACTUAL WEIGHT and work out the maximum dosage for each drug

(iii) Ensure that the patient has an obviously patent intravenous canula with some appropriate intravenous fluid infusing.

(iv) Ensure that the drugs are diluted appropriately and the necessary equipment is available and in good working condition.

(v) Delegate the responsibility of continual basic cardio respiratory monitoring (respiration and pulse) to a nurse or doctor.

(vi) Premedicate the child with atropine 0.02mg/Kg IV(0.2ml/kg)15 to 30 minutes before the beginning of the procedure.

(vii) Administer Ketamine 1 mg/Kg (0.2ml/kg) bolus and administer oxygen by mask at 5L/min.

(viii) Within 2 min, the child should be well sedated at which point consideration should be given to additional sedation if necessary with midazolam 0.05 mg/Kg (0.1ml/kg).

(ix) Where possible, infiltrate the operative site with lidocaine to a maximum of 4 mg/Kg(0.4ml/kg)

MANAGEMENT OF COMPLICATIONS

1. Bradycardia – Atropine 0.02 mg/Kg (0.2ml/kg) and repeat as necessary.
2. Cardiac arrest – Immediate CPR with chest compressions and Bag Valve Mask (Ambubag) ventilation. Administer adrenaline 0.1 mg/Kg IV (0.1ml/kg).
3. Inadequate sedation – Incrementally administer Ketamine boluses at 0.5 mg/Kg.(0.1ml/kg)

NB: Use of Local Anesthetics greatly reduces Ketamine/benzodiazepine requirements.

4. Excessive sedation – Ensure that the patient has a clear patent airway and is breathing well. Assist ventilation with a Bag Valve Mask bag if necessary. Monitor the pulse and Blood pressure every 5 minutes.

5. Convulsions – These will usually be due to local anesthetic toxicity. Manage with Midazolam at 0.15mg/kg (0.3ml/kg).

POST SEDATION CARE

1. Place the child in a lateral position and confirm adequate ventilation.

2. Administer oxygen by mask until the child is awake.

3. Neuropsychiatric manifestations may be managed with IV midazolam 0.05 mg/Kg.

4. Remember that the child will experience postoperative pain and prevent it if necessary.

5. Allow the child to feed once he/she is fully awake.

6. In case of intrathecally administered drugs let the patient be flat for (2-)4 hours

Adapted from the MTRH paediatric oncology manual September 2010

APPENDIX VII

PROCEDURE FOR TAKING A BLOOD SAMPLE FOR CBC

1. Sterilise the skin with spirit(>70% alcohol) with alcohol swab at the antecubital fossae or any visible peripheral vein
2. Allow the spirit to air-dry
3. Use a new sterile needle or cannula for venous puncture and collect 2-3 milliliters of blood in a standard EDTA anti-coagulated sample collection bottle.
4. Safely discard the needle and complete a laboratory request form.
5. Sample transported to the laboratory within 15 minutes.
6. Analysis was done using the flow cytometry method by Mindray BC 5500 5-diff and Mindray BC 3000 3-diff machines that produced 27 and 18 parameters respectively.
7. The results was made available within 60 minutes.

APPENDIX VIII

Assent form

I, Dr. Ahoya Phinehas Ademi, resident in the Department of Child Health and Paediatrics, Moi University, am carrying out a study on Response to Induction Chemotherapy in Paediatric Acute Lymphoblastic Leukemia at Moi Teaching and Referral Hospital, Eldoret.

The study involves follow up of a child during the period of getting the initial part of treatment lasting six weeks and documenting all findings during this period. This will involve documenting all tests done and physical findings during the six weeks. No additional investigations will be ordered for the purposes of this study.

There will be no direct benefits of participating in this study. Study subjects will be accorded same quality of management as non-study subjects.

All information obtained in this study will be treated with utmost confidentiality and shall not be divulged to any unauthorized person.

Participation in this study is voluntary, there is freedom to refuse to take part or withdraw at any time.

Sign or make a mark if you agree to take part in the study

Study subject: Investigator: Date:

…….. ……. …….

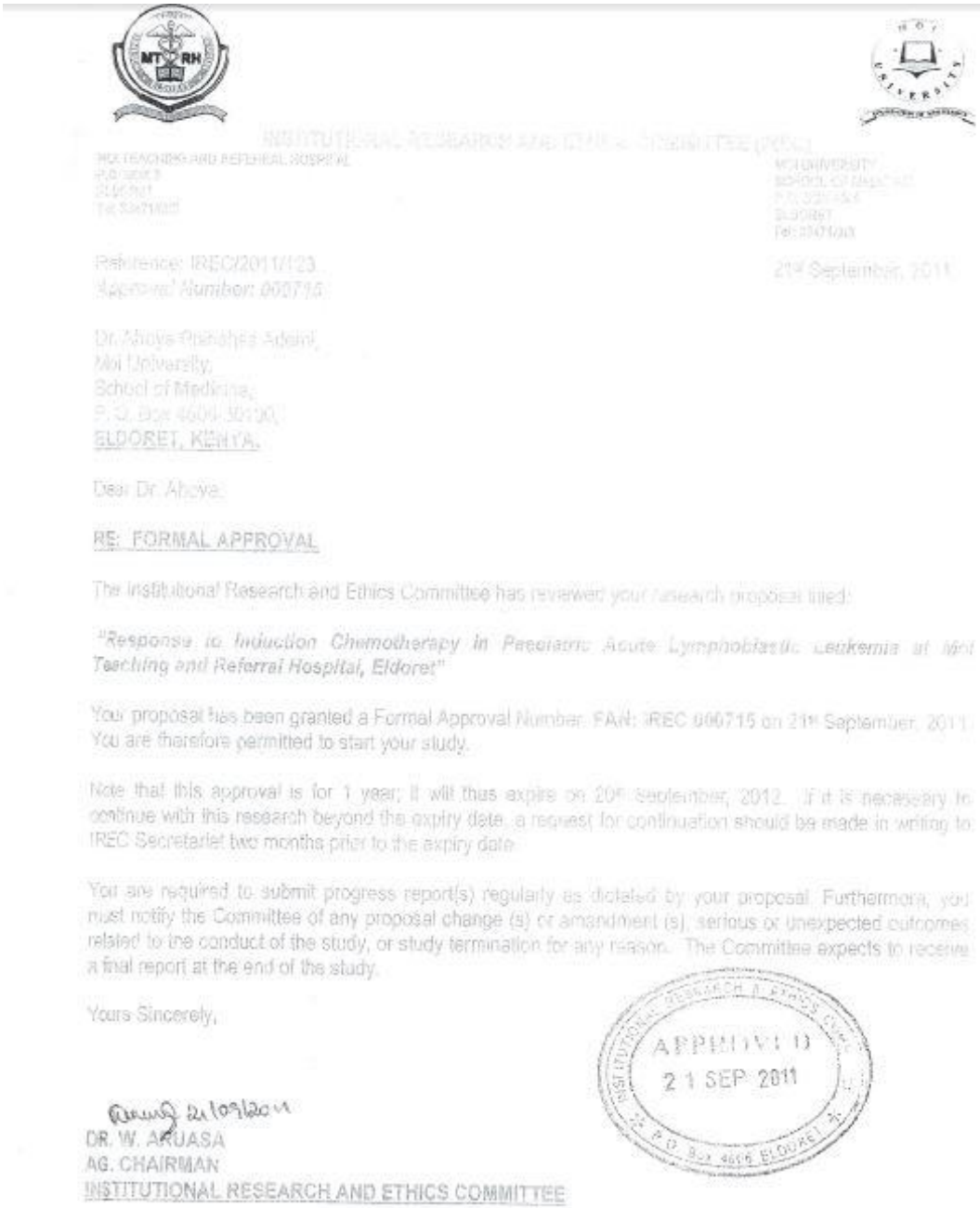
APPENDIX IX

HUMAN RESOURCE

Staff	**Role**	**Station**
Investigator /researcher	Write the proposal, data collection, analysis and write the study report.	Moi University, School of Medicine, Department of Child-Health and Paediatrics.
Supervisors	Guide on proposal writing, supervise during data collection, analysis and thesis writing.	Moi University, School of Medicine, Department of Child-Health and Paediatrics.
Research assistant	Assist in data collection	Moi University, School of Medicine, Department of Child-Health and Paediatrics.
Biostatistician	Assist in sample size calculation and data analysis.	Moi University, school of Public health, Department of Epidemiology and Preventive Medicine.

APPENDIX X

IREC APPROVAL



INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

MOI TEACHING AND REFERRAL HOSPITAL
P.O. BOX 3
ELDORET
TEL 33471/2/3

MOI UNIVERSITY
SCHOOL OF MEDICINE
P.O. BOX 4606
ELDORET
Tel: 33471/2/3

Reference: IREC/2011/123
Approval Number: 000715

21st September, 2011

Dr. Moya Phanphes Adeni,
Moi University,
School of Medicine,
P. O. Box 4606- 30100,
ELDORET, KENYA.

Dear Dr. Moya,

RE: FORMAL APPROVAL

The Institutional Research and Ethics Committee has reviewed your research proposal titled:

"Response to Induction Chemotherapy in Paediatric Acute Lymphoblastic Leukemia at Moi Teaching and Referral Hospital, Eldoret"

Your proposal has been granted a Formal Approval Number: FAN: IREC 000715 on 21st September, 2011. You are therefore permitted to start your study.

Note that this approval is for 1 year; it will thus expire on 20th September, 2012. If it is necessary to continue with this research beyond the expiry date, a request for continuation should be made in writing to IREC Secretariat two months prior to the expiry date.

You are required to submit progress report(s) regularly as dictated by your proposal. Furthermore, you must notify the Committee of any proposal change (s) or amendment (s), serious or unexpected outcomes related to the conduct of the study, or study termination for any reason. The Committee expects to receive a final report at the end of the study.

Yours Sincerely,

APPROVED
21 SEP 2011

21/09/2011

DR. W. ARUASA
AG. CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

cc: Director - MTRH
Dean - SOM
Dean - SPH
Dean - SOD

APPENDIX XI

LETTER FROM MTRH TO DO STUDY

MOI TEACHING AND REFERRAL HOSPITAL

Telephone: 2033471/2/3/4
Fax: 61749
Email: director@mtrh.or.ke

P. O. Box 3
ELDORET

Ref: ELD/MTRH/R.6/VOL.II/2007

21st September, 2011

Dr. Ahoya Phinehas Ademi,
Moi University,
School of Medicine,
P. O. Box 4606 – 30100,
ELDORET.

RE: APPROVAL TO CONDUCT RESEARCH AT MTRH

Upon obtaining approval from the Institutional Research and Ethics Committee (IREC) to conduct your research proposal titled:

"Response to Induction Chemotherapy in Paediatric Acute Lymphoblastic Leukemia at Moi Teaching and Referral Hospital, Eldoret."

You are hereby permitted to commence your investigation at Moi Teaching and Referral Hospital.

21/09/2011

DR. WILSON ARUASA
AG. DIRECTOR
MOI TEACHING AND REFERRAL HOSPITAL

CC - Deputy Director (CS)
- Chief Nurse
- HOD, HRISM