The Clinicopathological Profile of Diffuse Large B-Cell Lymphoma At Kenyatta National Hospital

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF A FELLOWSHIP IN MEDICAL ONCOLOGY,

UNIVERSITY OF NAIROBI

OYIRO OMONDI PETER

MB.Ch. B, MMED INT. MEDICINE (UON),

STUDENT FELLOW

SECTION OF HAEMATOLOGY AND ONCOLOGY, DEPARTMENT OF CLINICAL MEDICINE AND THERAPEUTICS,

SCHOOL OF MEDICINE,

UNIVERSITY OF NAIROBI.

DECLARATION

Declaration of Originality Form

OYIRO PETER OMONDI, H113/5854/2017
MB.Ch. B, MMED INTERNAL MEDICINE UoN)
SECTION OF HAEMATOLOGY AND ONCOLOGY, DEPARTMENT OF CLINICAL MEDICINE AND THERAPEUTICS, SCHOOL OF MEDICINE, UNIVERSITY OF NAIROBI

DECLARATION

1. I understand what Plagiarism is and I am aware of the University's policy in this regard
1. I declare that this thesis is my original work and has not been submitted elsewhere for examination, award of a degree or publication. Where other people's work or my own work has been used, this has properly been acknowledged and referenced in accordance with the University of Nairobi's requirements.
2. I have not sought or used the services of any professional agencies to produce this work.
3. I have not allowed, and shall not allow anyone to copy my work with intention of passing it off as his/her own work
4. I understand that any false claim in respect of this work shall result in disciplinary action in accordance with the University Plagiarism Policy

Signature…….

Date…….

Certificate of Authentication

This is to certify that this thesis is the original work of Dr. Oyiro Peter Omondi, Student Fellow in the Medical Oncology Fellowship Program, in the Department of Clinical Medicine and Therapeutics, registration number H113/5854/2017, the University of Nairobi. The research was carried out at the University of Nairobi, Kenyatta National Hospital, and University of Siena, Italy. It has not been presented elsewhere for award of a degree.

Professor Nicholas A. Othieno-Abinya MB.Ch. B, MMed, FRCP

Medical Oncologist and Professor of Medicine

Department of Clinical Medicine and Therapeutics

University of Nairobi.

Signed……Date……....

Supervisors:

DR. N.A. Othieno-Abinya- MB.Ch. B, MMed, FRCP

Medical Oncologist and Professor of Medicine

Department of Clinical Medicine and Therapeutics

University of Nairobi.

Signed ……Date …….

Prof. Lorenzo Leoncini, MD

Department of Medical Biotechnology

Director of Pathological Anatomy Division

University of Siena, Italy.

Signed ……  **… Date …….**

Nyagol Joshua, PhD

Department of Human Pathology

Unit of Immunology,

University of Nairobi.

Sign…… Date……

Dedication

This work is dedicated to my late parents: Mr. Rafael Oyiro Owor and Mrs. Rosbela Awor Oyiro for their invaluable sacrifice to enable me to attain this level of education.

Acknowledgements

To my God Almighty and to all the people who made this work possible and successful. Thank you! I particularly want thank Prof Nicholas O. Abinya and Prof Walter O. Mwanda who introduced me to the field of Hematology-Oncology and have been my main source of encouragement, guidance and mentorship. My sincere gratitude also goes to Prof. Lorenzo Leoncini for assisting with immunohistochemistry and molecular studies and Dr Joshua Nyagol for tirelessly guiding me through the whole process of the Dissertation. I also wish to thank Dr. Andrew O. Odhiambo for his valuable support and guidance. My sincere appreciation also goes to the staff at the Haemato-Oncology Clinic 23 of KNH for their great support, my colleagues for their steadfast encouragement and support. Last but not least, the Lymphoma patients whose consent enabled the study to be successful.

Finally, I am grateful to the University of Nairobi for allowing me to pursue this Fellowship in Medical Oncology and supporting my research interests.

Table of content

List of Tables

List of Figures

List of Abbreviations

ABC	Activated B cell
GCB	Germinal Center B cell
NHL	Non Hodgkins Lymphoma
R-CHOP	Rituximab, Cyclophosphamide, Doxorubicin, Vincristine, Prednisone
CD	Cluster of differentiation
COO	Cell of Origin
KNH	Kenyatta National Hospital
IPI	International Prognostic Index
NCCN	National Comprehensive Cancer Network
CR	Cure rate
ABC	Activated B cell
GCB	Germinal Center B cell
NHL	Non Hodgkins Lymphoma
R-CHOP	Rituximab, Cyclophosphamide, Doxorubicin, Vincristine, Pr
NOS	Not otherwise specified
CD	Cluster of differentiation
COO	Cell of Origin
KNH	Kenyatta National Hospital
IPI	International Prognostic Index
NCCN	National Comprehensive Cancer Network
CR	Cure rate
PEL	Primary Effusion Lymphoma
LDH	Lactate dehydrogenate
WHO	World health organization
EBV	Epstein Barr Virus
EBER	Epstein-Barr encoding region
HHV-8	Human Herpes Virus 8
PEL	Primary Effusion Lymphoma
LYG	plasmablastic lymphoma
MCD	Multicentric Castleman Disease
GEP	Gene expression profiling
PMBCL	Primary Mediastinal B Cell Lymphoma
IRF4/MUM 1	interferon regulatory factor 4/multiple myeloma oncogene 1
HL	Hodgkins Lymphoma
NLPHL	Nodular lymphocyte predominant Hodgkins Lymphoma

Abstract

Background*:* High grade Lymphoma such as diffuse large cell lymphoma (DLBCL) is a major cause of morbidity and mortality in developing countries. DLBCL is increasingly understood to be a heterogeneous disease with genetic, biologic, and clinical variants that have an important impact on clinical outcomes. There are three subtypes of DLBCL: Germinal Centre B-cell like DLBCL, Activated B-cell like DLBCL and Primary Mediastinal DLBCL. Whereas the association between the various subtypes and outcome is known, there is paucity of local data that examines the clinicopathological profile of DLBCL especially in an HIV endemic region. These data could provide more information on the association between DLBCL subtypes and in-depth understanding of the biology of DLBCL in the HIV setting.

Objectives: The aim of this study was to determine the clinicopathological profile of DLBCL classify DLBCL diagnosed at the Kenyatta National Hospital based on the Hans' algorithm and to correlate with the demographic and clinical characteristics of the patients.

Study design: This was a descriptive cross-sectional study

Study area: Kenyatta National Teaching and referral hospital

Materials and methods**:** Eighty-two patients diagnosed with diffuse large B cell lymphoma were consecutively recruited from among patients with non-Hodgkin's Lymphomas managed at the Kenyatta National Hospital. The paraffin embedded tissue blocks were retrieved from the archives at the various Pathology laboratories after relevant ethical and administrative approvals. Haematoxylin and eosin slides were prepared and reviewed to confirm the previous diagnosis and to classify the tumors. Immunohistochemical expression for various lymphoma markers were assessed and scored. Findings were correlated with relevant demographic, pathologic and clinical data.

Data management and analysis: Data was collected via written paper forms. After verification, data was then entered into a Microsoft Excel worksheet, and thereafter imported into the statistical analysis software for data management and analysis. Continuous data such as age was presented using means and respective standard deviations (SD) while categorical variables such as gender, treatment outcome were presented as percentages. Bivariate comparisons such as comparisons of by Germinal center DLBCL vs. Non-Germinal center DLBCL and Gender (Male vs. Female) was done using chi square or fishers' exact tests for categorical variables and t-test for continuous variables as appropriate. Univariable Logistic regression analysis to assess for demographic and clinical factors associated with Germinal

center DLBCL was done reporting the odds ratios (OR) and 95% Confidence Intervals. Stata version 15.1 (Stata Corp, College Station, Texas) was used for all statistical analyses. All statistical tests were evaluated at the 5% level ($p<0.05$) for statistical significance.

Results: Clinicopathological and immunophenotypic characteristics of 82 patients with diffuse large B-cell lymphoma (DLBCL) were examined. The mean age was 43.9 years (SD= 13.7) while the Median age was 43 (17-71years). The M: F was 1.4:1.

Based on immunophenotyping with anti-CD10, bcl-6, and MUM1 antibodies, the cases were categorized as GCB (CD10+ or CD10–, bcl-6+, MUM1+) or non-GCB phenotype. Forty-Five (54.9%) of the patients had GCB type while Thirty-Seven (45.1%) were of non-GCB origin. Age and ECOG performance status were the only clinical characteristics significantly associated with cell of origin. Older age was significantly associated with development of GCB subtype of DLBCL OR 1.45(1.03-2.04) p=0.032 and on univariate analysis and OR 1.67(1.07-2.52) P=0.023 on multivariate analysis.

Conclusions and recommendation: These results imply that cell of origin determination using immunohistochemistry on paraffin embedded tissue blocks has yielded important information that may predict the outcome of patients with non-Hodgkin B cell lymphomas in KNH. Generally, the patients studied had a poor CR rate (37%). Several factors including lack of timely chemotherapy seem to be responsible for this. Evaluation of prognostic or predictive biomarkers in the management of DLBCL, such as the COO, within prospective clinical trials will be important in the future.

CHAPTER ONE

1.0 Introduction

Diffuse large B-cell lymphoma comprises the largest proportion of the non-Hodgkin's lymphoma and comprises (30-40% of NHL) worldwide (1, 2). Whereas DLBCL has traditionally been identified and managed uniformly, it is now recognized as a heterogeneous entity with regards to its clinical, morphological and immunophenotypic profiles. The main treatment modality for patients with DLBCL for over a decade still remains combination immunochemotherapytherapy (3) with cure rates of 50-60% and Complete remission (CR) achieved after initial therapy in about 70%-95% of patients (4). The prognosis is, however; poor for those patients who develop primary disease progression or relapse. It is against this background that there is critical need to profile DLBCL and identify characteristics possibly responsible for the poor prognosis. Identifying these specific subgroups of patients is an effective strategy aimed at improving treatment outcomes (5), (3).

Most studies investigating the heterogeneity of DLBCL have focused on clinical and or morphological features without significant prognostic impact (5). For instance, the prognostic impact of certain morphological subtypes such as immunoblastic lymphoma remains to be investigated. There is also poor reproducibility of the clinical and histopathological criteria thus making it a challenge to formulate standard prognostic algorithm (5, 6). With the application of immunophenotypic markers used in various algorithms such as the Hans Criteria, DLBCL can be further be subclassified into three patterns namely: the germinal center (GC), activated B-cell (ABC) and primary mediastinal B cell DLBCL (7).

Investigators have also revealed lymphomas expressing the MYC translocation as well as those harboring additional "second hits" with translocations involving BCL2 or BCL6 referred to as "double hit" or "double expressed" lymphomas. MYC translocation is a known negative predictor of survival outcomes in DLBCL and is more commonly identified in the GC subtype (8). Double-hit lymphoma is reported at a frequency of about 15% of patients with NHL. Its characterized by chromosomal rearrangement involving CMYC and BCL2 or less frequently, BCL6 (9, 10). Since DLBCL subtypes are not identifiable through histological evaluation, it is necessary to perform further investigations such as IHC and fluorescent in-situ hybridization (FISH) to identify them. FISH is however, more expensive and not available for routine use. Therefore, there are efforts to standardize the identification

of certain subtypes of DLBCL such as the "Double expressed" or "double hit" lymphomas through protein expression on IHC.

The main aim of the study was therefore to classify DLBCL using the Hans immunohistochemical algorithm. It remains unclear the prevalence of the various categories of DLBCL in our setting and the significance on the outcome of patients with DLBCL. Furthermore, much is not known about the distribution of these subtypes in the background of HIV. Assessment of DLBCL subtypes therefore provides further information to aid in the development of effective strategies to diagnose and prognosticate DLBCL in our setting. The study also investigated the possible relationship between the subtypes and age, sex, IPI score, laboratory characteristics such as LDH, Ki-67, and HIV status.

CHAPTER TWO

2.0 Literature Review

2.1. Epidemiology of DLBCL

Non-Hodgkin's Lymphoma is a heterogenous group of lymphoid neoplasms of which DLBCL comprises about 30-40 percent. Morphologically, it features a diffuse proliferation of atypical large B cells with vesicular nuclei, prominent nucleoli and basophilic cytoplasm that typically express pan-B-cell markers such as CD 20, CD19, CD22, and CD79a. Bcl 6 expression is noted in approximately 60% of the cases.

There is relatively higher incidence of DLBCL in patients with HIV/AIDS. In the USA for instance, 5.5% of DLBCL and about 20% of Burkitt's lymphoma cases occur in the background of HIV/AIDS (11). In Sub-Saharan Africa, its estimated that the incidence is about 30,000 cases of NHL each year, 50% of which are HIV associated. Leoncini, et al in study of Lymphomas in Africa found Adult DLBCL comprising 55% of NHL while 7.5% of pediatric NHL. (12) In Kenya, NHL ranks eighth comprising 0.5% of all cancers (1, 13, 14).

The clinical presentation is that of a rapidly growing, non-painful mass in a lymph node or extranodal site. Constitutional "B" symptoms may be present. The median age at presentation is 55 years and its slightly more common in males than females (15). In 60% of cases the disease present in advanced stage. DLBCL present as extra nodal disease in up to 40% of cases (1). The commonly involved extranodal sites are the gastrointestinal tract (mainly stomach) and less frequently bone, breast, testes, central nervous system, thyroid, liver and kidney.

Rare cases may present with prominent sinusoidal involvement prompting the differential diagnosis of metastatic carcinoma. It has shown in several studies that 11-48% of DLBCL, NOS patients had bone marrow involvement by the lymphoma at presentation (16-18) and a concordant histology signified a worse prognosis compared with a discordant histology(17).

2.2 HIV and other Viral related DLBCL

These include the rare entities of plasmablastic lymphoma; Human Herpes Virus-8 associated associated Multicentric Castleman Disease, and primary effusion lymphoma (PEL). All are most commonly found in the background of immunosuppression due to HIV.

Plasmablastic lymphoma is mostly seen in HIV positive individuals but is also associated with other immunodeficiency states (19). It commonly presents as mass lesions in the oral cavity, but other extranodal sites can also be affected. This lymphoma is characterized by

plasmablastic differentiation with expression of CD38, CD79a (in most of cases), CD138, VS38, interferon regulatory factor 4/multiple myeloma oncogene 1 (IRF4/MUM1) and cytoplasmic immunoglobulin G (IgG). It also commonly show light chain restriction, and typically does not express Leukocyte Common Antigen (CD45) and other B-cell markers such as CD20 and paired box gene 5 (PAX5) (20). The lymphoma cells also commonly express epithelial membrane antigen (EMA) and CD30, and most of the cases are positive for EBV. Its however not associated with HHV-8. (21).

DLBCL transforming form HHV-8 associated MCD is most commonly seen in the background of HIV infection (22). In contrast to plasmablastic lymphoma, this entity commonly affects the lymph nodes and the spleen. The Human herpes virus-8 latency-associated nuclear antigen-1(LANA-I) is often demonstrated in the neoplastic cells in this entity. HHV-8 associated MCD large B-cell lymphoma is negative for EBV coded RNAs (EBER) and CD79a but is positive for CD20. This expression pattern is reciprocal to the plasmablastic lymphoma (1).

PEL mostly arises in the background of HIV infection. Immunostaining for LANA-1 and in-situ hybridization for EBER demonstrate universal presence of HHV-8 and frequent presence of EBV in the neoplastic cells. Patients typically present with lymphomatous effusions of one body cavity (pleural, pericardial, and peritoneal) with no mass lesion. The prognosis is poor. The lymphoma cells exhibit a highly abnormal immunophenotype with frequent loss of expression of B-cell antigens including CD19, CD 20, CD22 and CD79a (23). Its however characterized by the expression of CD45, IRF4/MUM-1, and CD138. PEL demonstrates a plasmablastic gene expression profile (24).

2.3 DLBCL specific to anatomic sites

DLBCL associated with certain specific anatomic sites may have unique biologic, clinical and prognostic characteristics. They include primary CNS DLBCL, primary cutaneous type DLBCL, Leg type, primary mediastinal and intravascular DLBCL. These entities are not generally associated with EBV. Primary CNS DLBCL are the intracebral or intraocular DLBCL that are not associated with systemic disease or immunodeficiency (25). Primary mediastinal DLBCL arises from the thymic B cells and frequently manifests as a mediastinal mass in young females. It has favorable prognosis (26) and can share some of the clinical, histologic, and immunophenotypic characteristics with nodular sclerosing HL (26). Intravascular DLBCL is rare and is confined to the vascular lumens (27). Clinical presentation is protean and is secondary to the occlusion of small blood vessels of various

organs by the lymphoma cells. It has recently been observed that IVBCL come in two subtypes: a classical subtype with frequent skin and CNS involvement and a second type associated with hemophagocytic syndrome, high incidence of hepatosplenic and bone marrow involvement, cytopenia and absence of skin involvement.

2.5 Molecular subtypes of DLBCL

In addition to morphology, further molecular studies such as gene expression profiling methods (GEP) can distinguish three entities of DLBCL such as: the germinal Center-B-cell (GCB), the activated B-cell (ABC) and Primary mediastinal DLBCL. The GCB group is postulated to have a better prognosis.(28, 29). These subgroups are also associated with different patterns of cytogenetic abnormalities with t(14;18) and gains of 12q12 more commonly seen in the GCB subtype (30, 31) where as 3q27 (BCL6) translocations are commonly seen in PMBCL and the ABC subtypes (30). GEP is not currently available for routine clinical practice but there have been successful attempts at simulating the GEP classification using immunohistochemistry (IHC) (32, 33). GEP has also been performed in CD5+ DLBCL with a series of genes identified to distinguish CD5+ DLBCL from CD5- DLBCL and Mantle cell Lymphoma(MCL) (34).

Alizadeh et al in 2000 employed the GEP method to study the various subtypes of DLBCL. (35). They used complimentary microarrays to examine the genetic features of normal versus malignant lymphoid cells (35). Based on their results there were two main types described. One type was noted to harbor genes like those observed in normal lymph node germinal center B cells hence designated the GCB while the other group showed high expression of the activated B-cell (ABC) genes. Patients with ABC experienced significantly poor outcomes to CHOP chemotherapy regimen compared to those with GCB DLBCL.

The Leukemia Lymphoma Molecular Profiling Project (LLMPP) later expanded on molecular subtyping of DLBCL by applying the microarray technology to a bigger sample size (28). In addition to the two entities, they identified a third entity with mixed expression of genes named "type 3". This subtype was later described as primary mediastinal DLBCL with poor outcomes like the ABC group. Several studies later found this group to have features of primary mediastinal DLBCL (36). (37).

Further evidence reveal that in ABC lymphoma, the activation of Nuclear factor-κβ through the chronic B-cell receptor signaling and aberrant Toll-like receptor signaling leads to the blocking of maturation beyond the plasmablastic phase (38). On the other hand it is

postulated that GCB DLBCL arise from a similar mechanism of differentiation block but beyond the germinal center B-cell (38). Even though as a group GCB DLBCL is generally considered to have slightly better outcomes following immunochemotherapeutic, hidden diversity within this group has also been demonstrated. For instance, those patients with translocations involving MYC and BCL2; the so called double hit lymphomas, have poor prognosis even though they are almost exclusively of GCB origin.

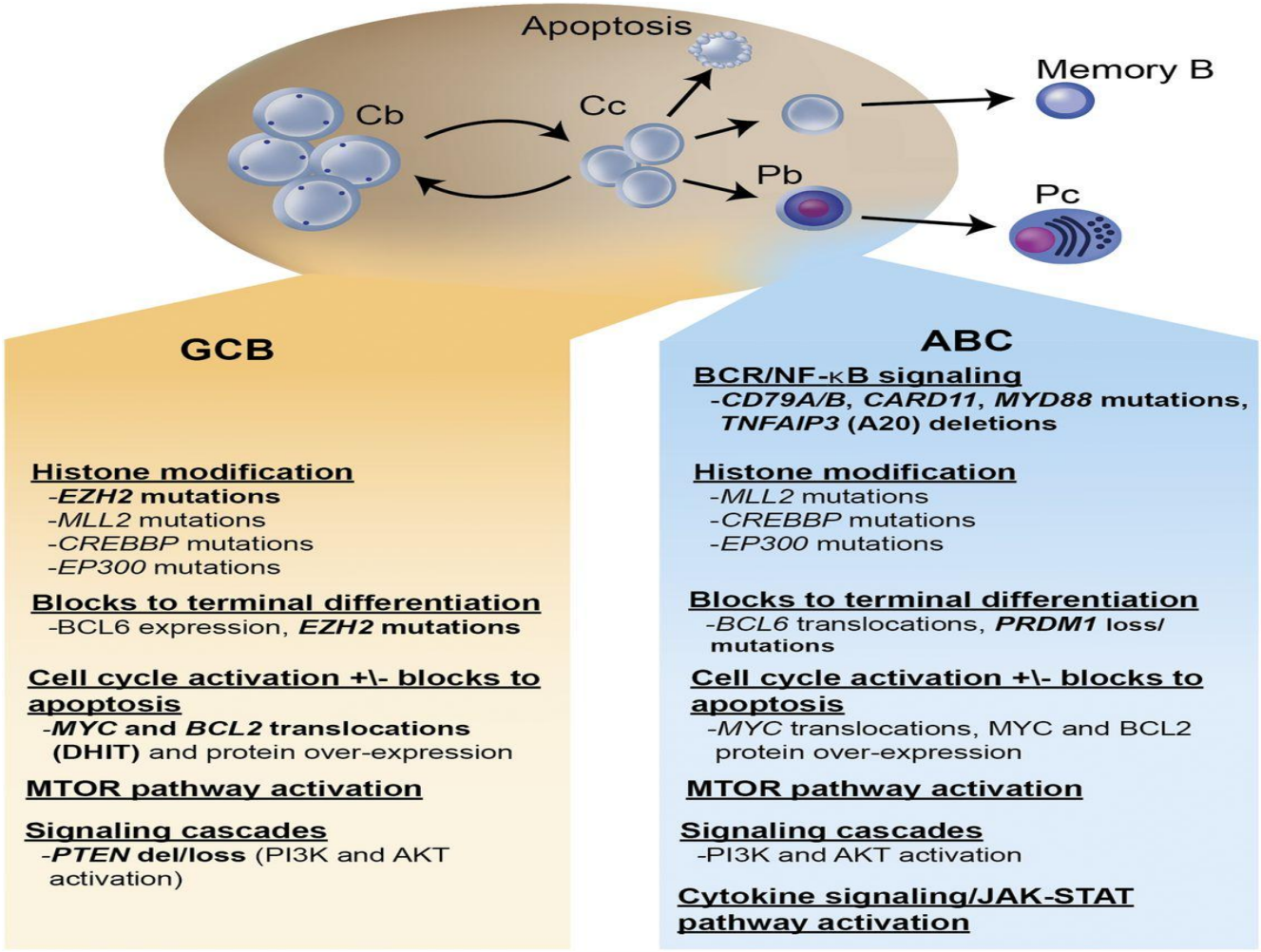


Figure 1: Key oncogenic pathways in DLBCL

Cb, centroblasts; Cc, centrocyte; Pb, plasmablast; Pc, plasma cell; DHIT, double-hit lymphoma; del, deletion; BCR, B-cell receptor.(39)

2.5 Role of DLBCL molecular subtypes in predicting Treatment outcome

There are several studies that have demonstrated a link between DLBCL subtypes and response to treatment. Currently there are also several clinical trials on treatment targets that are postulated to be effective in one or the other of the subgroups. (40, 41). Molina et al studied young patients with DLBCL treated based on cell of origin separation determined by IHC algorithms. They found minimal survival benefit with the intensified chemotherapy regimen such as R-ACVBP over R-CHOP among the ABC subtype of DLBCL. They concluded that IHC algorithms could be applied as a prognostic feature in the selection of patients with DLBCL with possible benefit from an intensified upfront treatment (41). Accurate assays for determining the various subtypes therefore need to be developed to enable proper patient selection. Ultimately these markers will be useful in guiding decisions in the routine workup and management of patients with DLBCL (28). Rosenwald et al studied 240 patients with DLBCL. In this study they used the Lymphochip microarray technique to assess the molecular sub classification of DLBCL. The study also demonstrated that the ABC-type was associated with poorer response to treatment with chemotherapy. Other studies in North America and Europe involving about 414 DLBCL patients, Lenz and colleagues demonstrated using univariate analysis that although Rituximab based chemotherapy regimen such as R-CHOP improves outcomes generally, the non-GCB subtype still had a poorer prognosis with approximately 45% three year progression-free survival as compared to about 74% for the GCB-type (6).

Currently, there are a number of IHC algorithms such as Hans *et al*,(7) Choi *et al*,(33); Meyer *et al*,(42) that have successfully reproduced the GEP classification.

The contribution of HIV infection on DLBCL subtypes has not been studied extensively. Some studies have not demonstrated any difference in the outcome among the various DLBCL subtypes. Chadbum et al investigated the significance of molecular subtype in HIV related DLBCL. In their study, biopsy specimens were subjected to immunophenotyping in an effort to identify the GCB, ABC or primary mediastinal DLBCL and correlate these with the outcome. In contrast to what is the case with HIV-negative DLBCL, they did not find any clinical relevance in HIV associated DLBCL (43) (44). There is probably no significant difference in tumor histogenesis between HIV negative and positive DLBCL. The knowledge of the role of tumor biology in HIV-associated lymphomas could be of value in identifying and development of personalized treatment to improve the outcome in DLBCL (44, 45). For instance, in non-HIV associated DLBCL, the activated B-cell subtype has been associated with poorer outcomes prognosis. This may be in part associated with the constitutive

activation of the pathophysiological pathways such as nuclear factor kappa B pathway, with downward signaling pathway involving CARD-11, BCL-10, and MALT-1 (46).

2.6 Prognostic Parameters in DLBCL

The International Prognostic Index (IPI) is for the past decade been the basis for the prognosis in patients with DLBCL managed with chemotherapy for the past two decades (47). The original IPI has been revised and still confirmed the prognostic significance even in the Rituximab era. Even though enhanced NCCN-IPI (48) appears to better separate low and higher risk groups, the original IPI is still widely used in clinical practice. The IPI has also been recently found to particularly predict increased risk of Central nervous system involvement.

The IPI was developed in 1993 based on the clinical features of about 1000 patients who had been treated with chemotherapy such as CHOP (49). It comprises five clinical features which include age, lactate dehydrogenase (LDH), number of extranodal sites, Ann Arbor stage and Eastern Cooperative Oncology Group (ECOG) performance status. These characteristics were applied in the International NHL prognostic factors project, in 1993 to risk stratify DLBCL and with these factors, distinct risk categories with significant prognostic impact were identified. By Characterizing patients based on the numerical strength of the prognostic factors as low (0-1 factor, 35% of patients), low-intermediate (2 factors, 27% of patients), high-intermediate (3 factors, 22%), or high (4-5 factors, 26%) the predicted five year overall survival were approximately 70%, 50%, 40% and 25%, respectively.

The introduction immunotherapy, Rituximab, to the chemotherapy backbone has translated into better overall survival across all the risk groups particularly the high risk group (50, 51). In a study conducted by the The Groupe d'Etude de Lymphome d'Adultes (GELA) comparing CHOP to R-CHOP in elderly patients with DLBCL, the five year overall survival of about 60% with R-CHOP compared to 45% with CHOP alone demonstrated the value of adding Rituximab to CHOP (52, 53). The IPI again was used in this study as well to distinguish the various prognostic groups. Some studies have questioned the ability of the IPI to distinguish between the various risk groups, particularly in the high risk group (51). Pooled data from three major European clinical trials however demonstrated a different picture asserting the role of the standard IPI in discriminating even between the risk groups. Several other studies have however demonstrated the role of IPI in distinguishing outcomes even in the high-risk groups. In the MabThera International Trial (MInT) of 380 patients, the study of

dose-escalated regimen of CHOP plus Etoposide (MegaCHOEP) trial, 72 patients and the CHOP -Rituximab for patients older than age 60 years (RICOVER-60) trial, 610 patients (50) that enrolled adult DLBCL patients treated with R-containing regimens, demonstrated a five year OS in the IPI-defined high-risk group of approximately 50%. The IPI is therefore still significant even in the immunochemoterapy era.

Several strategies have been tried to improve the IPI scoring. These have largely focused on addition of new parameters to the original index, reclustering the standard IPI or specific focus on the older population. These approaches have only resulted into incremental benefits. Other than the IPI, there is still a lack of rigorous prognostic methods for initial risk stratification in clinical practice.

2.7 Use Immunohistochemical Algorithms for prognostication

In parallel to the IPI score, there have been several efforts to determine the biological diversity of DLBCL for prognosis using immunophynotypical techniques. IHC markers can distinguish DLCBL into various subtypes with prognostic significance. One such algorithm is the Hans Immunohistochemical model that utilizes three markers namely CD10, BCL-2, BCL-6 and MUM1/IRF4. This model is validated as a marker to define the DLBCL subtypes with about 80% concordance with the GEP(7). The Hans algorithm divides DLBCL into three main categories: Germinal Center (GC), and non-GC DLBCL. Additional IHC algorithms have since then been proposed to predict the clinical behavior of DLBCL (54).

In the rituximab era, the clinical significance of DLBCL sub-classification has been controversial. Some studies have failed to demonstrate any difference in outcomes between germinal and non-germinal center denovo DLBCL treated with R-CHOP(55) while other have found that better response with enhanced regimens such as R-EPOCH. (56).

2.8 Study rationale

The determination of its subtypes has notably progressed given improving availability and affordability of immunophenotypic markers applicable in clinical use.

There are various prognostic tools that have been generated, validated and are currently used in the management of patients with this lymphoma. The critics of the IPI score maintain that it fails to capture the biological heterogeneity of DLBCL hence cannot assist in identifying potential therapeutic targets. There is therefore is critical need to profile DLBCL subtypes and clinical characteristics possibly peculiar to our setting. In the absence of such knowledge, the development of effective intervention strategies to improve DLBCL outcomes in our setting will likely remain problematic.

The determination of the COO in DLBCL has allowed the separation of the distinctive DLBCL subtypes in most of the developed countries, but such an approach has not yet been employed in Kenya to guide management.

The use of Immunohistochemistry algorithms has been shown to be practical alternative to gene expression profiling (GEP) which is arguably the preferred method of classifying DLBCL. Immunohistochemistry of course has its major challenges in clinical practice such as lack of standardization of laboratory techniques across institutions to maintain the reproducibility of tests results over time, and to establish a consensus in IHC scoring by pathologists.

Research question

What is the clinical and immunophenotypic profile of diffuse large B Cell Lymphoma at Kenyatta National Hospital?

2.9 Objectives of the Study

Objectives of the Study

General Objective:

To determine the clinicopathological profile of DLBCL managed at Kenyatta National Hospital

Specific Objectives:

i. To document the DLBCL subtypes seen at KNH using Hans's immunohistochemistry algorithm.

ii. To correlate the DLBCL subtypes with clinical and demographic characteristics

iii. To correlate the various molecular subtypes of DLBCL with clinical characteristics and outcome

CHAPTER THREE

3.O Materials and Methods

3.1 The Study design: This was a cross sectional descriptive study

3.2 The Study Setting:

The study was conducted at Kenyatta National Teaching and referral Hospital, a tertiary teaching and referral hospital located in Nairobi, Kenya. It was established in 1900 and is the largest hospital in the Eastern and Central Africa and has a capacity of 2000 beds. It is also the teaching hospital for various universities and colleges among them the University of Nairobi. It also serves as a referral hospital for Kenya and East Africa. It runs general and specialized clinics and in-patient's services in surgical, medical, obstetrics and gynaecology, ophthalmology and paediatrics.

The haematology clinic is carried out every Monday. The venue is usually the Kenyatta National Hospital clinic number 23. About 80 patients are seen in the clinic every week out of which about 3 are diagnosed with DLBCL. The patients' records are available in the health information records office.

KNH/UON haematology and pathology laboratories have the capacity for morphological analysis and evaluation of DLBCL subtypes.

3.3 Study Population:

Patients diagnosed with NHL, aged 13 years and above on treatment and regular follow-up at KNH.

3.4 Sampling:

All consecutive patients with documented diagnosis of DLBCL on treatment and follow-up were recruited into the study. The sample size calculation below was used to estimate the sample size from the population of DLBCL patients attending the clinic:

Sample size calculation (The Daniel's formula 1999 for finite population(57)

$n = Nz^2pq/ (E2 (N-1) + z^2pq)$

Where

- N (population size) =100,
- Z (confidence level) = 1.96
- E (±error) = 0.05,
- p (prevalence) = 0.3
- Q (1-p) = 0.7
- n = 80

3.5 Selection of Patients

Inclusion Criteria:

Patients included into the study were those aged 13 years and above with a diagnosis of DLBCL and signed informed consent or assent.

Exclusion criteria

Those patients who declined to give signed informed consent, and below 13 years of age were excluded from the study.

3.6 Methodology

The patients were recruited sequentially until the targeted sample size is reached. Study proforma was used to document patients' clinical, demographic and laboratory characteristics. Only patients who gave signed informed consent or assent were allowed to participate into the study.

3.6.1 Demographic and Clinical details

The patient's demographic details such as age, sex, residence, education, occupation was taken and documented in a standardized data extraction proforma. Clinical characteristics including Ann Arbor stage at diagnosis, bone marrow status, lactate dehydrogenase (LDH) levels, HIV status was documented and IPI scoring done for each patient. Treatment history including chemotherapy and radiotherapy history was also documented.

IPI Subtyping

Samples with an IPI value of 0-1 were classified as low IPI, those with IPI values of 2-3 were classified as intermediate IPI, those with IPI values 4-5 were classified as high IPI. For some of the samples one or more of the IPI variables were missing. If for a given sample the value for the missing variable would not change the IPI group call (e.g. depending on the value of the missing variable the IPI value would be either 2 or 3) then the sample would be included in the IPI analysis as a member of that IPI group. However, if the missing value could make a difference (e.g. between 3 and 4) then that sample was excluded from all analyses involving IPI.**(14)**

3.6.2. Laboratory Procedures

For each study case, the file was perused for results of the staging including LDH and bone marrow aspirate/trephine reports documented. Tissue blocks, and slides were retrieved for histological review at the UON/KNH pathology laboratory. Suspected cases of DLBCL were shipped to collaborator laboratory at the University of Siena-Italy using both institutions (UON/KNH) material transfer agreement for immunophenotyping and molecular analyses. The following markers were analyzed: CD20, CD3, CD10, BCL6, BCL2 and MUM-1. The procedures for Immunohistochemistry and RNA scope are annexed.

The study got approval from the KNH/UoN Ethics and regulatory Committee. The permission to ship samples to Siena, Italy was granted by the Director of Medical Services, Ministry of Health (copy annexed).

3.6. Laboratory results and interpretation

Serum LDH was interpreted as normal, high or low. The reference range is 95-200 units per Litre. Serum LDH is a marker of cell turn over hence a proxy to the disease burden/bulk and aggressiveness. It can also be raised in other disease states such as megaloblastic anaemia, hemolysis among others that were not investigated in this study.

The immunohistochemical markers such as CD20, 3, 5, 10, 45, Bcl-2, 6, c-myc, were interpreted as positive depending on the cytoplasmic/ nuclear positivity for the antibody against the marker. For each marker, a positive and negative control will be run concurrently as per the recommendation of the manufacturer's specification for the antibody.

The cases that express CD10 were considered germinal centre type. Those that are CD10 negative and bcl6 negative were considered of non- germinal centre origin. However, those cases that were CD10 negative but bcl6 positive, were subjected to MUM1 testing and if positive then were classified as of non- germinal centre origin like those that are both CD10 and Bcl- 6 negative (figure 2).

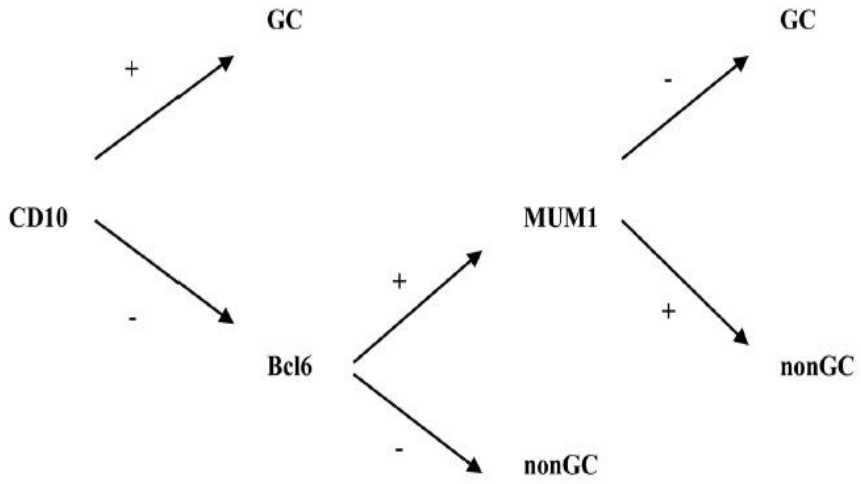
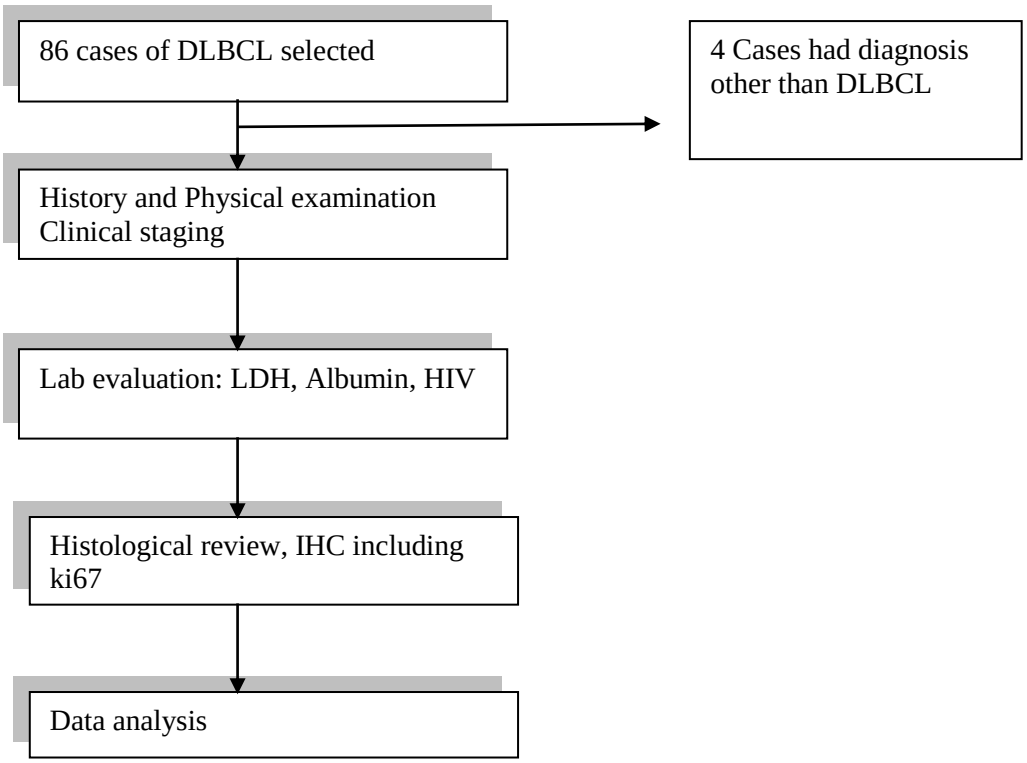


Figure 2: Hans Immunohistochemical Algorithm

Study variables

The independent variables were age, sex, morphological classification, while dependent ones were DLBCL subtype, IPI score, clinical stage at diagnosis and HIV status.

Patients Flow chart



3.7. Quality Assurance:

The standard operating procedures were adhered to**.** In the pre-analytical phase, we ensured that the request/order forms were written for the tests required with proper patient identification. The patient unique identification number was written on the request form and the sample collection container. Sufficient amount of sample i.e. 3mls for LDH were drawn in the appropriate container in proper order using standard procedures and delivered to the laboratory within the 3-4 hours. The machines used for analysis are calibrated according to manufactures recommendations.

The tissue blocks were retrieved for histology and for immunohistochemistry, adhering to kit manufactures' instructions and procedure. This was done both locally and at the pathology laboratory at the University of Siena Italy.

The KNH/UON haematology and biochemistry laboratories run daily internal and external quality control on all tests. The machines/equipment are calibrated daily using commercially available kits.

3.8: Data Management and Statistical Analysis

Data was collected via written paper forms. After verification, data was then entered into a Microsoft Excel worksheet, and thereafter imported into the statistical analysis software for data management and analysis. Continuous data such as age was presented using means and respective standard deviations (SD) while categorical variables such as gender, treatment outcome were presented as percentages. Bivariate comparisons such as comparisons of by Germinal center DLBCL vs. Non-Germinal center DLBCL and Gender (Male vs. Female) was done using fishers' exact tests for categorical variables and t-test for continuous variables as appropriate. Univariable Logistic regression analysis to assess for demographic and clinical factors associated with Germinal center DLBCL was done reporting the odds ratios (OR) and 95% Confidence Intervals. Stata version 15.1 (Stata Corp, College Station, Texas) was used for all statistical analyses. All statistical tests were evaluated at the 5% level ($p<0.05$) for statistical significance.

4.0. Ethical Considerations

Informed consent/assent form were signed by the patients or parent (for patients below 18 years of age) as established and consistent with the policies of research of clinical type for government of Kenya ministry of health/research science and technology and UON/KNH ethics and Research Committee. Confidentiality was maintained throughout the study. The study findings were communicated to the clinicians for timely and continued management of the involved subjects and those seen in future. Those who declined informed consent or assent were not victimized. After Ethics Committee approval, authority to use the medical records in Kenyatta National Hospital was sought and granted from the KNH Research Office and the Medical Record In-Charge in Kenyatta National Hospital.

5.0. Results

The study was conducted between May 2018 to Aug 2018. The characteristics of the study population are presented below:

5.1. Demographic characteristics of the study population

Eighty-six (86) patients with the diagnosis of high grade lymphomas aged 17 years and above were screened and subsequently, 82 were enrolled into the study. The other four were found to have a diagnosis other than DLBCL. Over half 48 (57.8%) were males, while the females were 34 (42.2%) of the study population. The mean age of the population was 43.9 years (SD = 13.7) interquartile range of 34-56. Median age was 43 with range between 17-71 years. The mean age for the females was 43±11 years while that for the males was 44±12years. The male to female ratio was 1.4:1.

Table 1 :Sex distribution of the study population

Descriptive Table	n (%)
SEX	
F (n=34)	34 (41.5)

M (n=48)	48 (58.5)
Age (years) Mean (SD)	43.9 (13.7)

5.2 Histomorphological diagnosis and clinical characteristics

In the studied population, 47 (58%) and 21 (30.4%), were initially reported as DLBCL and High-Grade NHL histology, respectively (**Table 3**). The common sites at diagnosis in the study population were lymph nodes (54%), Gastrointestinal system 32%, sinonasal/ tonsillar and nasopharyngeal (6%), oral cavity (4%) and breast (3%).

Majority of the participants, 63(76.8%), had more than one ECOG/PS. Similarly, so, a majority, 68 (89.5%), had more than the Upper limit of normal LDH category. Slightly over half, 28 (56%), had bone marrow involvement. Under half of the participants, 37 (45.1%), had no extranodal site. One quarter, 20 (24.4%), of the participants had bulky disease and just over half, 45 (54.9%), had B symptoms present. Most of the participants, 57 (72.2%), had Ann Abor Stage III-IV Category. Majority, 51 (67.1%), had a high IPI score category with almost similar proportion,53 (67.9%), being HIV negative. (**Table 3)**

Table 2 :Histomorphological features of the study population

Diagnosis	n (%)
ANAPLASTIC LARGE B CELL LYMPHOMA (n=3)	3 (3.7)
DLBCL (n=47)	47 (58)
DLBCL-THR (n=1)	1 (1.2)
DLBCL/ BL (n=4)	4 (4.9)
HIGH GRADE NHL (n=25)	25 (30.4)
PLASMABLSTIC LYMPHOMA (n=1)	1 (1.2)

Table 3 :Clinical characteristics of the study Population

ECOG/PS Category	
<=1 (n=19)	19 (23.2)
>1 (n=63)	63 (76.8)
LDH Category	
> Upper limit of normal (n=68)	68 (89.5)
Low (<=222) (n=8)	8 (10.5)
Bone marrow involvement	
Involved (N=22)	22 (44)
Not Involved (N=28)	28 (56)
Extranodal site number count	
0 (n=37)	37 (45.1)
1 (n=26)	26 (31.7)
2 (n=15)	15 (18.3)
3 (n=4)	4 (4.9)
Bulky Disease	
ABSENT (n=62)	62 (75.6)
PRESENT (n=20)	20 (24.4)
B Symptoms	
Absent (N=37)	37 (45.1)
Present (N=45)	45 (54.9)
Ann Abor Stage	
I-II (n=22)	22 (27.8)
III-IV (n=57)	57 (72.2)

IPI Score Category	
High (3-5) (n=51)	51 (67.1)
Low (0-2) (n=25)	25 (32.9)
HIV status	
NEG (n=53)	53 (67.9)
POS (n=25)	25 (32.1)

5.3 Treatment History

Half of the participants, 42 (51.2%), had CHOP 6 to 8 treatment given to them while 12 (14.6%) received RCHOP. Only 6 (7.3%) received Intrathecal treatment. Thirty patients (39.5%) achieved Complete Remission (CR). Seventeen (22.4%) had PR. Six (7.9%) had progressive disease while only one patient had stable disease as at the time of this analysis. The most common salvage treatments were ICE 7 (41.2%) and R-ICE 3 (17.6%). **Table 4**

Table 4 :Treatment History of the Study population

Treatment	n (%)
CHOP (n=49)	49 (59.7)
CHOP 1 (n=1)	1 (1.2)
CHOP 2 (n=1)	1 (1.2)
CHOP 4 (n=3)	3 (3.7)
CHOP 8 (n=11)	11 (13.4)
CHOP 8+IT (n=4)	4 (4.9)
NO CHEMO (n=1)	1 (1.2)
R-CHOP (n=3)	3 (3.7)
R-CHOP 2 (n=1)	1 (1.2)
R-CHOP 8+IT (n=1)	1 (1.2)
R-CHOP+IT (n=1)	1 (1.2)
R-CHOP 6 (n=6)	6 (7.3)
1st line treatment	
Treatment CHOP 6 to 8	
CHOP 6-8 (n=49)	49 (59.7%)
Treatment R-CHOP	
RCHOP (n=12)	12 (14.6%)

Treatment Intrathecal	
Intrathecal Methotrexate (IT) (n=6)	6 (7.3%)
Salvage treatment	
D-HAP (n=5)	5 (26.3)
ICE (n=7)	7 (36.8)
MACOP-B (n=1)	1 (5.2)
R-DHAP (n=2)	2 (11.5)
R-ICE (n=4)	4 (21.0)

Table 5 Laboratory results of the study population

Antigen	**n(%)**
CD 20	
Neg (n=7)	7 (8.8)
Pos (n=73)	73 (91.2)
BCL 6	
Neg (n=43)	43 (78.2)
Pos (n=12)	12 (21.8)
BCL 2	
Neg (n=9)	9 (47.4)
Pos (n=10)	10 (52.6)
CD3	
Neg (n=36)	36 (87.8)
Pos (n=5)	5 (12.2)
CD10	
Neg (n=37)	37 (45.7)
Pos (n=44)	44 (54.3)
MYC(IHC)	
Neg (n=1)	1 (33.3)
Pos (n=2)	2 (66.7)
MYC(FISH)	

Pos (n=2)	2 (100)
CD45	
Neg (n=2)	2 (4.1)
Pos (n=47)	47 (95.9)
EBER	
Pos (n=2)	2(100)
IRF4/MUM1	
Neg (n=5)	5 (41.7)
Pos (n=7)	7 (58.3)
Ki-67%	
<=60(n=40)	19(32%)
>60	40(68%)

The following markers were analyzed in the 82 patients: CD20, CD10, BCL2, BCL-6, and MUM-1**. Table 5**. Most of DLBCL cases expressed CD10 (44 positive cases of 82 evaluable cases). CD 20 expression was seen in 73 (91.2%), while 12 (21.8%), had BCL 6 positive results and 10 (52.6%), were BCL2 positive. MUM-1 expression was seen in 7 out 12.

Using the Hans' algorithm, forty-five cases were classified as GCB while 37 cases were Non-Germinal Center DLBCL.

The GCB subtype of DLBCLs included 21females and 24 males while the non-GCB 13 females and 24 males. There was a statistically significant difference in the mean age between the GCB (46.8 ±12.5 years) and the non-GCB (40.2± 14.3 years) groups of patients (P=0.02 t-test). The 2 subtypes of DLBCL did not differ about any other clinical features. **Table 6 and 7.**

5.4 Clinical biologic correlation

The proportion of males with non-GCB DLBCL was higher than that among GCB type without any significance, 64.9% vs. 53.3%, p value =0.292. The mean age was significantly higher among the Germinal center DLBCL, 46.9 years (SD=12.6) vs. 40.2 years (14.3), p value = 0.029. The proportion of participants with ECOG/PS greater than 1 among Non-Germinal center DLBCL group was higher than that among Germinal center DLBCL without any statistical significance, 32 (86.5%) vs. 31 (68.9%), p value =0.06. The proportions of participants with low or high LDH categories were similar among the two groups, p value =1.000. Similarly, the proportions of participants with extranodal site counts were similar among the two groups. A similar pattern was seen in Ann Abor Stage Category, p value = 0.879. The proportion of participants with a high IPI Score (3-5) among non- GCB DLBCL group was lower than that among GCB DLBCL without any statistical significance, 22

(62.9%) vs. 29 (70.7%), p value =0.466. There were no statistical differences among the non-GCB DLBCL group and GCB DLBCL with respect to HIV status and the outcome, all p values > 0.05. **Table 6, 7**

Table 6 :Relationship between Clinical Characteristics and Gender

Variables	Female (n=34)	Male (n=48)	P value
Age in years Mean (SD)	43.1 (11.8)	44.5 (15)	0.652
ECOG/PS Category			
<=1 (n=19)	10 (29.4)	9 (18.8)	0.261
>1 (n=63)	24 (70.6)	39 (81.2)	
LDH Category			
> Upper limit of normal (n=68)	28 (93.3)	40 (87)	0.376
Low (<=222) (n=8)	2 (6.7)	6 (13)	
Ki-67			
<=60	9(47.4)	10(52.6)	0.53
>60%	16(40)	24(60)	
Extranodal site number count			
0 (n=37)	12 (35.3)	25 (52.1)	0.161
1 (n=26)	14 (41.2)	12 (25)	
2 (n=15)	5 (14.7)	10 (20.8)	
3 (n=4)	3 (8.8)	1 (2.1)	
Ann Abor Stage Category			
I-II (n=22)	9 (28.1)	13 (27.7)	0.964
III-IV (n=57)	23 (71.9)	34 (72.3)	
IPI Score Category			

High (3-5) (n=51)	22 (73.3)	29 (63)	0.351
Low (0-2) (n=25)	8 (26.7)	17 (37)	
HIV status			
NEG (n=53)	22 (66.7)	31 (68.9)	0.835
POS (n=25)	11 (33.3)	14 (31.1)	
OUTCOME			
CR (n=30)	9 (28.1)	21 (47.7)	0.388
DEAD (n=4)	2 (6.2)	2 (4.5)	
ON TX (n=18)	9 (28.1)	9 (20.5)	
PD (n=6)	2 (6.2)	4 (9.1)	
PR (n=17)	10 (31.2)	7 (15.9)	
SD (n=1)	0 (0)	1 (2.3)	

5.5 The relationship between clinical characteristics and gender

The mean ages were not different by gender, p value = 0.652. The proportion of participants with ECOG/PS greater than 1 was higher among Males than Females, however, without any statistical significance, 39 (81.2%) vs. 24 (70.6%), p value = 0.261. The proportions of participants with low or high LDH categories were almost similar among the genders, p value =0.376. Males had fewer sites of extranodal sites of involvement compared to the females, but this was not significant, p = 0.161. There was also no significant difference in Ann Abor Stage Category, by gender, p value = 0.964. Females had a higher proportion of high IPI Score (3-5) than among males but without any statistical significance, 22 (73.3%) vs. 29 (63%), p=0.351. More males had CR than females, but the difference was not statistically significant, 21 (47.7%) vs. 9 (28.1%), p = 0.388. **Table 6**

Table 7: Relationship between Cell of Origin and clinical characteristics

Variables	Non-Germinal center DLBCL (n=37)	Germinal center DLBCL (n=45)	P value
SEX			
F (n=34)	13 (35.1)	21 (46.7)	0.292
M (n=48)	24 (64.9)	24 (53.3)	
Age in years Mean (SD)	40.2 (14.3)	46.9 (12.6)	0.029
ECOG/PS Category			
<=1 (n=19)	5 (13.5)	14 (31.1)	0.06
>1 (n=63)	32 (86.5)	31 (68.9)	
LDH Category			
> ULN (n=68)	31 (88.6)	37 (90.2)	1
LLN (<=222) (n=8)	4 (11.4)	4 (9.8)	
Extranodal site number			
0 (n=37)	15 (40.5)	22 (48.9)	0.727
1 (n=26)	14 (37.8)	12 (26.7)	

2 (n=15)	6 (16.2)	9 (20)	
3 (n=4)	2 (5.4)	2 (4.4)	
Ann Abor Stage			
I-II (n=22)	10 (27)	12 (28.6)	0.879
III-IV (n=57)	27 (73)	30 (71.4)	
IPI Score Category			
High (3-5) (n=51)	22 (62.9)	29 (70.7)	0.466
Low (0-2) (n=25)	13 (37.1)	12 (29.3)	
HIV status			
Neg (n=53)	25 (69.4)	28 (66.7)	0.793
Pos (n=25)	11 (30.6)	14 (33.3)	
Treatment Outcome			
CR (n=30)	12 (36.4)	18 (41.9)	0.928
DEAD (n=4)	1 (3)	3 (7)	
Ongoing treat. (n=18)	9 (27.3)	9 (20.9)	
PD (n=6)	3 (9.1)	3 (7)	
PR (n=17)	8 (24.2)	9 (20.9)	
SD (n=1)	0 (0)	1 (2.3)	

5.6 Logistic regression analysis of predictive features

Clients had increased odds of having a Germinal center DLBCL for every 10 years increase in age in both univariable (OR=1.45 (95% CI: 1.03-2.04), p = 0.032) and multivariable analysis (OR=1.67 (95% CI: 1.07-2.52), p=0.023). Male study participants compared to the females had lower odds of having a Germinal center DLBCL in both univariable and multivariable models, however without any statistical significance. Participants with Ann-Abor Stage III-IV compared to stage I-II had lower odds of having Germinal center DLBCL in both univariable and multivariable (OR=0.09 (95% CI: 0.01-1.49), p= 0.093) models, however without any statistical significance. Those with ECOG/PS category more than one compared those with one or less had statistically significantly lowered odds of having Germinal center DLBCL in the multivariable model (OR=0.05 (95%CI: 0.01-0.40), P=0.004). Patients with Lower LDH values were compared to those with more than upper limits, had lower odds of having a having Germinal center DLBCL in both the univariable and multivariable model without any statistical significance. Participants with low IPI Score (0-2) compared to those with high score (3-5) had statistically significantly lower odds of

having a Germinal center DLBCL in multivariable model (OR=0.70 (95% CI: 0.27- 0.83), p = 0.038). There was a slight increase in the odds of having a Germinal center DLBCL among the HIV positive participants compared to the HIV negative ones in both the univariable and multivariable model, however, without any statistical significance. With regard to the outcome, using CR as the reference groups, the odds of having a Germinal center DLBCL was highly increased among those who died (OR=1.99 (95%: 0.19-21.57)), however without any statistical significance, p=0.568. **Table 8**

Table 8: Logistics regression analysis

	Univariable		Multivariable	
Outcome: Germinal center DLBCL vs. Non-Germinal center DLBCL	OR (95% CI)	P value	OR (95% CI)	P value
Age (per 10 years)	1.45 (1.03-2.04)	0.032*	1.67 (1.07-2.52)	0.023*
Gender				
Female	1		1	
Male	0.62 (0.25-1.51)	0.293	0.94 (0.30-2.94)	0.915
Ann-Arbor Stage Category				
I-II	1		1	
III-IV	0.93 (0.35-2.48)	0.879	0.09 (0.01-1.49)	0.093
ECOGPS Category				
≤1	1		1	
>1	0.35 (0.11-1.08)	0.067	0.05 (0.01-0.40)	0.004*
LDH Category				
>upper limit of normal	1		1	
Low (≤222)	0.84 (0.19-3.63)	0.813	0.61 (0.09-4.38)	0.624

IPI Score Category				
High (3-5)	1		1	
Low (0-2)	0.70 (0.27-1.83)	0.467	0.04 (0.01-0.83)	0.038*
Extra-nodal site number category				
≤1	1			
>1	1.17 (0.42-3.31)	0.763		
HIV status				
Negative	1		1	
Positive	1.14 (0.44-2.96)	0.793	1.10 (0.35-3.52)	0.866
Outcome				
CR	1			
Dead	1.99 (0.19-21.57)	0.568		
ON TX	0.67 (0.21-2.16)	0.5		
PD	0.67 (0.11-3.87)	0.651		
PR	0.75 (0.23-2.49)	0.639		
SD	-	-		

CHAPTER FIVE

6. Discussion

In addition to clinical heterogeneity demonstrated by the IPI, recent publications have shown molecular heterogeneity within DLBCL that influences prognosis (1, 28).

In this study we present data on comprehensive clinical, morphological, immunohistochemical study of a cohort of DLBCL managed at the KNH, making special reference to prognostic factors and markers that have been reported to define biologic subgroups of DLBCL. This study demonstrated that majority of DLBCL were of Germinal Center origin. Forty-five (54.9%) of the lymphoma patients in this study population had GCB type while 37 (45.1%) were of non-GCB origin. Our findings are consistent with other findings mostly in the developed countries where the GCB subtype has also been demonstrated in the majority of the DLBCL. A study by Choi *et al*, for instance, concurs with our findings.(33) They studied 74 cases of DLBCL and reported that the majority 47(56%) were GCB DLBCL. In addition, similar observations was made that IHC algorithms are valuable tools that can be employed in the risk stratification of DLBCL (33). Other studies have, however, shown contrary results on the prevalence of DLBCL subtypes. For instance a study by Hans *et al* that employed the Hans' algorithm which include markers such as CD10,

BCL-6 and MUM-1 in a cohort of 152 DLBCL cases, found the 88(58%) of the DLBCL cases to be of the non-GCB subtype (7). In addition, they also found that the GCB subtype was associated with better prognosis. They study concluded that determining the cell of origin of DLBCL using immunostains is valuable in predicting survival, like the application of cDNA microarray. Currently, subtyping of DLBCL into GCB and non-GCB can be achieved with significant accuracy by immunohistochemical (IHC) algorithms such as the Han's method as used in the present study (7). Even though more patients with GCB got into CR as compared to the ones with non-GCB, this had no significance P=0.91.

Our finding of higher prevalence of the GCB DLBCL seems to be concordance with several other DLBCL studies mostly from North America and Western Europe (58), although our sample size was smaller.

The role of molecular sub-classification using IHC algorithms in risk stratification of DLBCL is currently well established based on the results of several independent studies (59, 60). However not all studies have confirmed the prognostic significance of the DLBCL subtypes when identified by IHC (Hans' algorithm).

We did not observe any significant association between the two subtypes and the clinical or demographic parameters such as sex, stage at diagnosis, IPI score, nodal or extranodal involvement or HIV status. In our opinion, this lack of significant association between the DLBCL subtypes and the other clinical characteristics including outcome in this study may, in part, be related to retrospective analysis of samples and management that incorporated different treatments.

Age and performance are among the five independent prognostic factors that have been incorporated into various prognostic models in DLBCL such as the IPI score used in this study. Age and ECOG performance status were the only clinical characteristics significantly associated with cell of origin. Older age was significantly associated with the development of GCB subtype of DLBCL, using both univariate, OR 1.45(1.03-2.04) p=0.032 and multivariate analysis, OR 1.67(1.07-2.52) P=0.023.

Several studies have demonstrated strong association between age, performance status and outcome with various chemotherapy regimens. The value of age and performance status in risk stratification of NHL including DLBCL was first demonstrated in the International NHL prognostic factor Index study in 1993. This was a project, involving 2031 patients, was designed to develop a model of predicting outcomes of patients with aggressive NHL on the basis of clinical characteristics before treatment (61). Several other studies have since re-

evaluated this model with age adjustment in the advent of newer treatment modalities most notably with the addition of Rituximab with similar outcomes (51, 62).

The DLBCL sub typing by IHC using the Hans Algorithm as used in the present study has not to the best of our knowledge, been reported before in Kenya (58, 60). Markers such as CD10 and bcl-6, are expressed in normal follicular germinal centers and are preferentially expressed in germinal center derived DLBCL. Most of our patients expressed both CD10 and bcl-6. Bcl-6 over expression in DLBCL has been recently associated with a better prognosis.(63). In our study, 12 out of 55 cases had bcl-6 expression (21.8%) with no significant association between bcl-6 expression and clinical parameters.

A study by Lossos and colleagues noted a strong predictive value for survival of bcl-6 protein and mRNA overexpression in DLBCLs. (64) They however had a small sample of only 30 patients who had bcl-6 expression. Another study by Bodoor et al also observed that a germinal center phenotype defined by CD10 and bcl-6 co-expression was significantly associated with good prognostic factors such as lower IPI.(65) However, this study had a smaller number of patients with advanced stage and high-risk IPI as compared to our current that involved both nodal and extranodal disease.

The transcription factor MUM1/IRF4 is a key member of the interferon regulatory factor (IRF) family of genes (66) that plays an significant function in the regulation of gene expression in response to signaling by various cytokines such as interferons.(67) In normal B cells, MUM1 and bcl-6 have mutual exclusive positivity (67). MUM1-positive lymphomas in our study were assigned to a non-GCB DLBCL. MUM-1 analysis was done in only 12 patients and was detected in seven (58.3%) all of which were also bcl6 positive.

Out of the 19 cases analyzed for bcl-2 protein over-expression, nine were found to have bcl-2 expression, two in the non-GCB and 7 in the GCB DLBCL. With the small number, we were unable to determine any significant association with clinical parameters or outcome. In other studies however, bcl-2 overexpression has been significantly associated with advanced disease and poor survival. (68).

In summary, other than age and ECOG performance status, the cell of origin, as assessed by immunophenotyping, was not associated with any other clinicopathological features of patients with DLBCL in our study population.

Our study therefore has set the stage for evaluation of treatment outcomes in DLBCL in well-designed prospective studies

The DLBCL demographics found in this study are generally consistent with age and sex distribution of as discussed in other studies. The finding that approximately 54% of the study population had extranodal sites presentation at diagnosis could be of therapeutic significance and comparable with the 30-40% extranodal presentation observed among DLBCLs in other studies such as in Germany (69), but not previously documented in Kenya. Wurzburg et al found the gastrointestinal tract as the commonest primary extranodal site followed by CNS. They also found out that extranodal involvement was common in the older age group. In Japan however, the extra-nodal presentation was higher (83.3%) in the young than (60.0%) in the older age-group(70). The pattern of extranodal site involvement was however comparable. Thus it seems that nodal and extranodal DLBCL, as well as DLBCL from different primary sites, are heterogeneous with regard to different biologic characteristics and prognostic implications (71).

Lymphoma classification still remains a challenge particularly to resource-constrained settings where further investigations as IHC, PCR and cytogenetics are not easily accessible. Thus, the WHO classification has been difficult to implement in such centers. As realized from the present study combined immunophenotyping and H & E staining, clearly improved diagnostic specificity and should be implemented routinely. Thus, the application of simple algorithms such as the Hans Algorithm used in this study can improve diagnostic accuracy and aid in the WHO classification in resource constrained settings.

The introduction antiretroviral therapy for HIV/AIDS has led to a significant reduction in the incidence of HIV/AIDS related lymphoma. (72, 73). Prospective findings in HIV associated DLBCL, have demonstrated that IHC analysis of DLBCL subtype predicts both lymphoma-specific and overall survival (74, 75).

In our study, sub-classification of HIV/AIDS related DLBCL into GCB or non-GCB type using the Hans' methods did not predict CR and was neither associated with significant clinical or demographic parameters. Various studies have reported higher incidence of lymphoma expressing markers such as CD10 and BCL-6 in HIV. (74, 76)(80)(74)

Our data confirm a slightly different distribution of antigen expression, with more frequent co-expression of both GCB cell antigens (CD10 and BCL-6) and a non-GCB cell marker (MUM-1). In this study, we found no predictive impact of most immunohistochemical markers. (77)

To the best of our knowledge, we are not aware of a biologic basis to suggest that the tumor histogenesis of HIV-associated DLBCL is fundamentally different from HIV negative

DLBCL. Indeed, several theories including immune deficiency and stimulation as well as virally driven histogenesis such as Epstein-Barr virus activation have been advanced as possible mechanisms.

In summary the observations of the present study have revealed the following features of DLBCL patients hitherto not described in our setting: That the majority of DLBCL are of the Germinal Center origin and older patients with poor ECOG performance status are more likely to be of GCB subtype. There is no impact of HIV in the development of either subtype of DLBCL, but HIV associated DLBCL have higher proliferative index. We found no significant association between the various subtypes and clinical characteristics

6.1 Conclusion

These results imply that cell of origin determination using Immunohistochemistry on paraffin embedded tissue blocks has yielded important information that may predict the outcome of patients with non-Hodgkin B cell lymphomas in KNH. Forty-five (54.9%) of the lymphoma patients in this study population had GCB type while 37 (45.1%) were of non-GCB origin. Other than age and performance status, none of the other clinical parameters had significant correlation with DLBCL subtypes.

6.2 Recommendation and suggestions for further studies

This study has laid the ground for further studies that may focus on the aspects of correlation between HIV and DLBCL histogenesis and between double hit, double expressed, or triple hit lymphomas and clinical outcome. Evaluation of prognostic and predictive biomarkers in the management of DLBCL, such as the COO, within prospective clinical trials will be important in the future. We recommend that the WHO classification of DLBCL be adhered to in the diagnostic workup.

6.3 Study Limitations and delimitations

Poor record keeping interfered with collection of information on staging and prognostic index details. Retrieval of tissue blocks from some laboratories was also a challenge.

Lack of uniform system for reporting and adherence to the WHO system of classification of DLBCL. Some cases were misdiagnosed as DLBCL.

7.0 References

1. Swerdlow S, Campo E, Harris N, Jaffe E, Pileri S, Stein H, et al. World Health Organization classification of tumours of haematopoietic and lymphoid tissues. 2008. WHO PRESSIARC.
2. Armitage JO. A clinical evaluation of the International Lymphoma Study Group classification of non-Hodgkin's lymphoma. Blood. 1997;89(11):3909-18.
3. Sehn LH, Donaldson J, Chhanabhai M, Fitzgerald C, Gill K, Klasa R, et al. Introduction of combined CHOP plus rituximab therapy dramatically improved outcome of diffuse large B-cell lymphoma in British Columbia. Journal of Clinical Oncology. 2005;23(22):5027-33.
4. Coiffier B, Thieblemont C, Van Den Neste E, Lepeu G, Plantier I, Castaigne S, et al. Long-term outcome of patients in the LNH-98.5 trial, the first randomized study comparing rituximab-CHOP to standard CHOP chemotherapy in DLBCL patients: a study by the Groupe d9Etudes des Lymphomes de l9Adulte. Blood. 2010;116(12):2040-5.
5. Coiffier B, Thieblemont C, Van Den Neste E, Lepeu G, Plantier I, Castaigne S, et al. Long-term outcome of patients in the LNH-98.5 trial, the first randomized study comparing rituximab-CHOP to standard CHOP chemotherapy in DLBCL patients: a study by the Groupe d'Etudes des Lymphomes de l'Adulte. Blood. 2010;116(12):2040-5.
6. Lenz G, Wright G, Dave S, Xiao W, Powell J, Zhao H, et al. Stromal gene signatures in large-B-cell lymphomas. New England Journal of Medicine. 2008;359(22):2313-23.
7. Hans CP, Weisenburger DD, Greiner TC, Gascoyne RD, Delabie J, Ott G, et al. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood. 2004;103(1):275-82.
8. Copie-Bergman C, Cuillière-Dartigues P, Baia M, Briere J, Delarue R, Canioni D, et al. MYC-IG rearrangements are negative predictors of survival in DLBCL patients treated with immunochemotherapy: a GELA/LYSA study. Blood. 2015;126(22):2466-74.
9. Aukema SM, Siebert R, Schuuring E, van Imhoff GW, Kluin-Nelemans HC, Boerma E-J, et al. Double-hit B-cell lymphomas. Blood. 2011;117(8):2319-31.
10. Barrans S, Crouch S, Smith A, Turner K, Owen R, Patmore R, et al. Rearrangement of MYC is associated with poor prognosis in patients with diffuse large B-cell lymphoma treated in the era of rituximab. Journal of clinical oncology. 2010;28(20):3360-5.
11. Shiels MS, Pfeiffer RM, Gail MH, Hall HI, Li J, Chaturvedi AK, et al. Cancer burden in the HIV-infected population in the United States. Journal of the National Cancer Institute.

2011;103(9):753-62.
12. Naresh KN, Raphael M, Ayers L, Hurwitz N, Calbi V, Rogena E, et al. Lymphomas in sub-Saharan Africa–what can we learn and how can we help in improving diagnosis, managing patients and fostering translational research? British journal of haematology. 2011;154(6):696-703.
13. Korir A, Okerosi N, Ronoh V, Mutuma G, Parkin M. Incidence of cancer in Nairobi, Kenya (2004–2008). International journal of cancer. 2015;137(9):2053-9.
14. Anderson J, Armitage J, Berger F, Cavalli F, Chan W, Close J, et al. A clinical evaluation of the International Lymphoma Study Group Classification of non-Hodgkin's lymphoma: a report of the Non-Hodgkin's Lymphoma Classification Project. Blood. 1997.
15. Shiels MS, Pfeiffer RM, Hall HI, Li J, Goedert JJ, Morton LM, et al. Proportions of Kaposi sarcoma, selected non-Hodgkin lymphomas, and cervical cancer in the United States occurring in persons with AIDS, 1980-2007. Jama. 2011;305(14):1450-9.
16. Campbell J, Seymour JF, Matthews J, Wolf M, Stone J, Juneja S. The prognostic impact of bone marrow involvement in patients with diffuse large cell lymphoma varies according to the degree of infiltration and presence of discordant marrow involvement. European journal of haematology. 2006;76(6):473-80.
17. Chung R, Lai R, Wei P, Lee J, Hanson J, Belch AR, et al. Concordant but not discordant bone marrow involvement in diffuse large B-cell lymphoma predicts a poor clinical outcome independent of the International Prognostic Index. Blood. 2007;110(4):1278-82.
18. Hodges GF, Lenhardt TM, Cotelingam JD. Bone marrow involvement in large-cell lymphoma: prognostic implications of discordant disease. American journal of clinical pathology. 1994;101(3):305-11.
19. Colomo L, Loong F, Rives S, Pittaluga S, Martínez A, López-Guillermo A, et al. Diffuse large B-cell lymphomas with plasmablastic differentiation represent a heterogeneous group of disease entities. The American journal of surgical pathology. 2004;28(6):736-47.
20. Delecluse H, Anagnostopoulos I, Dallenbach F, Hummel M, Marafioti T, Schneider U, et al. Plasmablastic lymphomas of the oral cavity: a new entity associated with the human immunodeficiency virus infection. Blood. 1997;89(4):1413-20.
21. Dong HY, Scadden DT, de Leval L, Tang Z, Isaacson PG, Harris NL. Plasmablastic lymphoma in HIV-positive patients: an aggressive Epstein-Barr virus-associated extramedullary plasmacytic neoplasm. The American journal of surgical pathology. 2005;29(12):1633-41.
22. Oksenhendler E, Boulanger E, Galicier L, Du M-Q, Dupin N, Diss TC, et al. High incidence of Kaposi sarcoma–associated herpesvirus–related non-Hodgkin lymphoma in patients with HIV infection and multicentric Castleman disease. Blood. 2002;99(7):2331-6.
23. Nador RG, Cesarman E, Chadburn A, Dawson DB, Ansari MQ, Sald J, et al. Primary effusion lymphoma: a distinct clinicopathologic entity associated with the Kaposi's sarcoma-associated herpes virus. Blood. 1996;88(2):645-56.
24. Klein U, Gloghini A, Gaidano G, Chadburn A, Cesarman E, Dalla-Favera R, et al. Gene expression profile analysis of AIDS-related primary effusion lymphoma (PEL) suggests a plasmablastic derivation and identifies PEL-specific transcripts. Blood. 2003;101(10):4115-21.
25. Cobbers J, Wolter M, Reifenberger J, Ring GU, Jessen F, An HX, et al. Frequent In activation of CDKN2A and Rare Mutation of TP53 in PCNSL. Brain pathology. 1998;8(2):263-76.
26. Boleti E, Johnson P. Primary mediastinal B-cell lymphoma. Hematological oncology. 2007;25(4):157-63.
27. Ferreri AJ, Dognini GP, Campo E, Willemze R, Seymour JF, Bairey O, et al. Variations in clinical presentation, frequency of hemophagocytosis and clinical behavior of intravascular lymphoma diagnosed in different geographical regions. haematologica. 2007;92(4):486-92.
28. Rosenwald A, Wright G, Chan WC, Connors JM, Campo E, Fisher RI, et al. The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. New England Journal of Medicine. 2002;346(25):1937-47.
29. Shipp MA, Ross KN, Tamayo P, Weng AP, Kutok JL, Aguiar RC, et al. Diffuse large B-cell lymphoma outcome prediction by gene-expression profiling and supervised machine learning. Nature medicine. 2002;8(1):68-74.
30. Iqbal J, Sanger WG, Horsman DE, Rosenwald A, Pickering DL, Dave B, et al. BCL2 translocation defines a unique tumor subset within the germinal center B-cell-like diffuse large B-cell

lymphoma. The American journal of pathology. 2004;165(1):159-66.
31. Bea S, Zettl A, Wright G, Salaverria I, Jehn P, Moreno V, et al. Diffuse large B-cell lymphoma subgroups have distinct genetic profiles that influence tumor biology and improve gene-expression-based survival prediction. Blood. 2005;106(9):3183-90.
32. Hans CP, Weisenburger DD, Vose JM, Hock LM, Lynch JC, Aoun P, et al. A significant diffuse component predicts for inferior survival in grade 3 follicular lymphoma, but cytologic subtypes do not predict survival. Blood. 2003;101(6):2363-7.
33. Choi WW, Weisenburger DD, Greiner TC, Piris MA, Banham AH, Delabie J, et al. A new immunostain algorithm classifies diffuse large B-cell lymphoma into molecular subtypes with high accuracy. Clinical cancer research. 2009;15(17):5494-502.
34. Kobayashi T, Yamaguchi M, Kim S, Morikawa J, Ogawa S, Ueno S, et al. Microarray reveals differences in both tumors and vascular specific gene expression in de novo CD5+ and CD5− diffuse large B-cell lymphomas. Cancer research. 2003;63(1):60-6.
35. Alizadeh AA, Eisen MB, Davis RE, Ma C, Lossos IS, Rosenwald A, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature. 2000;403(6769):503-11.
36. Rosenwald A, Wright G, Leroy K, Yu X, Gaulard P, Gascoyne RD, et al. Molecular diagnosis of primary mediastinal B cell lymphoma identifies a clinically favorable subgroup of diffuse large B cell lymphoma related to Hodgkin lymphoma. Journal of Experimental Medicine. 2003;198(6):851-62.
37. Shaffer III AL, Young RM, Staudt LM. Pathogenesis of human B cell lymphomas. Annual review of immunology. 2012;30:565-610.
38. Béguelin W, Popovic R, Teater M, Jiang Y, Bunting KL, Rosen M, et al. EZH2 is required for germinal center formation and somatic EZH2 mutations promote lymphoid transformation. Cancer cell. 2013;23(5):677-92.
39. Sehn LH, Gascoyne RD. Diffuse large B-cell lymphoma: optimizing outcome in the context of clinical and biologic heterogeneity. Blood. 2015;125(1):22-32.
40. Thieblemont C, Briere J, Mounier N, Voelker H-U, Cuccuini W, Hirchaud E, et al. The germinal center/activated B-cell subclassification has a prognostic impact for response to salvage therapy in relapsed/refractory diffuse large B-cell lymphoma: a bio-CORAL study. Journal of clinical oncology. 2011;29(31):4079-87.
41. Molina TJ, Canioni D, Copie-Bergman C, Recher C, Brière J, Haioun C, et al. Young Patients With Non–Germinal Center B-Cell–Like Diffuse Large B-Cell Lymphoma Benefit From Intensified Chemotherapy With ACVBP Plus Rituximab Compared With CHOP Plus Rituximab: Analysis of Data From the Groupe d'Etudes des Lymphomes de l'Adulte/Lymphoma Study Association Phase III Trial LNH 03-2B. Journal of Clinical Oncology. 2014;32(35):3996-4003.
42. Meyer PN, Fu K, Greiner TC, Smith LM, Delabie J, Gascoyne RD, et al. Immunohistochemical methods for predicting cell of origin and survival in patients with diffuse large B-cell lymphoma treated with rituximab. Journal of clinical oncology. 2010;29(2):200-7.
43. Chadburn A, Chiu A, Lee JY, Chen X, Hyjek E, Banham AH, et al. Immunophenotypic analysis of AIDS-related diffuse large B-cell lymphoma and clinical implications in patients from AIDS Malignancies Consortium clinical trials 010 and 034. Journal of Clinical Oncology. 2009;27(30):5039-48.
44. Dunleavy K, Little RF, Pittaluga S, Grant N, Wayne AS, Carrasquillo JA, et al. The role of tumor histogenesis, FDG-PET, and short-course EPOCH with dose-dense rituximab (SC-EPOCH-RR) in HIV-associated diffuse large B-cell lymphoma. Blood. 2010;115(15):3017-24.
45. Dunleavy K, Pittaluga S, Czuczman MS, Dave SS, Wright G, Grant N, et al. Differential efficacy of bortezomib plus chemotherapy within molecular subtypes of diffuse large B-cell lymphoma. Blood. 2009;113(24):6069-76.
46. Lenz G, Davis RE, Ngo VN, Lam L, George TC, Wright GW, et al. Oncogenic CARD11 mutations in human diffuse large B cell lymphoma. Science. 2008;319(5870):1676-9.
47. Chaganti S, Illidge T, Barrington S, Mckay P, Linton K, Cwynarski K, et al. Guidelines for the management of diffuse large B-cell lymphoma. British journal of haematology. 2016;174(1):43-56.
48. Zhou Z, Sehn LH, Rademaker AW, Gordon LI, LaCasce AS, Crosby-Thompson A, et al. An

enhanced International Prognostic Index (NCCN-IPI) for patients with diffuse large B-cell lymphoma treated in the rituximab era. Blood. 2014;123(6):837-42.
49. Shipp M, Harrington D, Anderson J, Armitage J, Bonadonna G, Brittinger G, et al. A predictive model for aggressive non-Hodgkin's lymphoma. New England Journal of Medicine. 1993;329(14):987-94.
50. Ziepert M, Hasenclever D, Kuhnt E, Glass B, Schmitz N, Pfreundschuh M, et al. Standard International prognostic index remains a valid predictor of outcome for patients with aggressive CD20+ B-cell lymphoma in the rituximab era. Journal of clinical oncology. 2010;28(14):2373-80.
51. Sehn LH, Berry B, Chhanabhai M, Fitzgerald C, Gill K, Hoskins P, et al. The revised International Prognostic Index (R-IPI) is a better predictor of outcome than the standard IPI for patients with diffuse large B-cell lymphoma treated with R-CHOP. Blood. 2007;109(5):1857-61.
52. Coiffier B, Lepage E, Brière J, Herbrecht R, Tilly H, Bouabdallah R, et al. CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. New England Journal of Medicine. 2002;346(4):235-42.
53. Feugier P, Van Hoof A, Sebban C, Solal-Celigny P, Bouabdallah R, Ferme C, et al. Long-term results of the R-CHOP study in the treatment of elderly patients with diffuse large B-cell lymphoma: a study by the Groupe d'Etude des Lymphomes de l'Adulte. Journal of Clinical Oncology. 2005;23(18):4117-26.
54. Chang C-C, McClintock S, Cleveland RP, Trzpuc T, Vesole DH, Logan B, et al. Immunohistochemical expression patterns of germinal center and activation B-cell markers correlate with prognosis in diffuse large B-cell lymphoma. The American journal of surgical pathology. 2004;28(4):464-70.
55. Nyman H, Adde M, Karjalainen-Lindsberg M-L, Taskinen M, Berglund M, Amini R-M, et al. Prognostic impact of immunohistochemically defined germinal center phenotype in diffuse large B-cell lymphoma patients treated with immunochemotherapy. Blood. 2007;109(11):4930-5.
56. Wilson WH, Dunleavy K, Pittaluga S, Hegde U, Grant N, Steinberg SM, et al. Phase II study of dose-adjusted EPOCH and rituximab in untreated diffuse large B-cell lymphoma with analysis of germinal center and post-germinal center biomarkers. Journal of Clinical Oncology. 2008;26(16):2717-24.
57. Naing L, Winn T, Rusli B. Practical issues in calculating the sample size for prevalence studies. Archives of orofacial Sciences. 2006;1(1):9-14.
58. Muris J, Meijer C, Vos W, van Krieken J, Jiwa N, Ossenkoppele G, et al. Immunohistochemical profiling based on Bcl-2, CD10 and MUM1 expression improves risk stratification in patients with primary nodal diffuse large B cell lymphoma. The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland. 2006;208(5):714-23.
59. Alizadeh AA, Eisen MB, Davis RE, Ma C, Lossos IS, Rosenwald A, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature. 2000;403(6769):503.
60. Wang K, Chen C, Shi P, Yu J, Tan J, Qian S, et al. Prognostic Value of Morphology and Hans Classification in Diffuse Large B Cell Lymphoma. Zhongguo shi yan xue ye xue za zhi. 2018;26(4):1079-85.
61. Project IN-HsLPF. A predictive model for aggressive non-Hodgkin's lymphoma. New England Journal of Medicine. 1993;329(14):987-94.
62. Zhou Z, Sehn LH, Rademaker AW, Gordon LI, LaCasce AS, Crosby-Thompson A, et al. An enhanced International Prognostic Index (NCCN-IPI) for patients with diffuse large B-cell lymphoma treated in the rituximab era. Blood. 2013:blood-2013-09-524108.
63. Lin J, Zheng Y, He H, Wang J, Yang Y, Chen D, et al. Clinicopathological Features and Prognostic Factors of DLBCL. Zhongguo shi yan xue ye xue za zhi. 2018;26(3):779-83.
64. Lossos I, Akasaka T, Martinez-Climent J, Siebert R, Levy R. The BCL6 gene in B-cell lymphomas with 3q27 translocations is expressed mainly from the rearranged allele irrespective of the partner gene. Leukemia. 2003;17(7):1390.
65. Bodoor K, Matalka I, Hayajneh R, Haddad Y, Gharaibeh W. Evaluation of BCL-6, CD10, CD138 and MUM-1 expression in diffuse large B-cell lymphoma patients: CD138 is a marker of poor prognosis. Asian Pacific Journal of Cancer Prevention. 2012;13(7):3037-46.
66. Gaidano G, Carbone A. MUM1: a step ahead toward the understanding of lymphoma histogenesis. Nature Publishing Group; 2000.

67. Gualco G, Weiss LM, Bacchi CE. MUM1/IRF4: a review. Applied Immunohistochemistry & Molecular Morphology. 2010;18(4):301-10.
68. Makino K, Nakamura H, Shinojima N, Kuroda J-i, Yano S, Mikami Y, et al. BCL2 expression is associated with a poor prognosis independent of cellular origin in primary central nervous system diffuse large B-cell lymphoma. Journal of neuro-oncology. 2018:1-7.
69. Ott G, Rosenwald A. Extranodal diffuse large B-cell lymphoma--an organotypic disease? Der Pathologe. 2007;28(1):29-35.
70. Yamauchi A, Fujita S, Ikeda J, Nakamichi I, Fukuhara S, Hino M, et al. Diffuse large B-cell lymphoma in the young in Japan: A study by the Osaka Lymphoma Study Group. American journal of hematology. 2007;82(10):893-7.
71. Lu J, Li X, Zhang P, Zhou X, Zhang T, Li X, et al. Nodal versus extranodal diffuse large B-cell lymphoma: comparison of clinicopathologic features, immunophenotype and prognosis. Zhonghua bing li xue za zhi= Chinese journal of pathology. 2007;36(7):470-3.
72. Engels EA, Biggar RJ, Hall HI, Cross H, Crutchfield A, Finch JL, et al. Cancer risk in people infected with human immunodeficiency virus in the United States. International journal of cancer. 2008;123(1):187-94.
73. Gibson TM, Morton LM, Shiels MS, Clarke CA, Engels EA. Risk of non-Hodgkin lymphoma subtypes in HIV-infected people during the HAART era: a population-based study. AIDS (London, England). 2014;28(15):2313.
74. Madan R, Gormley R, Dulau A, Xu D, Walsh D, Ramesh K, et al. AIDS and non-AIDS diffuse large B-cell lymphomas express different antigen profiles. Modern pathology. 2006;19(3):438.
75. Dunleavy K, Little RF, Pittaluga S, Grant N, Wayne AS, Carrasquillo JA, et al. The role of tumor histogenesis, FDG-PET, and short course EPOCH with dose-dense rituximab (SC-EPOCH-RR) in HIV-associated diffuse large B-cell lymphoma. Blood. 2010:blood-2009-11-253039.
76. Little RF, Pittaluga S, Grant N, Steinberg SM, Kavlick MF, Mitsuya H, et al. Highly effective treatment of acquired immunodeficiency syndrome–related lymphoma with dose-adjusted EPOCH: impact of antiretroviral therapy suspension and tumor biology. Blood. 2003;101(12):4653-9.
77. Bu R, Hussain AR, Al-Obaisi KA, Ahmed M, Uddin S, Al-Kuraya KS. Bortezomib inhibits proteasomal degradation of IκBα and induces mitochondrial dependent apoptosis in activated B-cell diffuse large B-cell lymphoma. Leukemia & lymphoma. 2014;55(2):415-24.

8.0 Appendix

Appendix I: Proforma/questionnaire

A. Sociodemographic Section

Date ……. /……../……..
Serial number……IP/OP number
Age(years)_____ Sex: Male.....Female.....
Occupation☐ 1. Employed 2. Unemployed 3. Self-employed 4. Other

Education level ☐ 1. Primary 2. Secondary 3. College 5. None

Marital Status: Married ☐ Single ☐ Divorced ☐ Separated ☐ NA ☐

Residence(county)……
Duration of follow-up…….

B. Clinical characteristics

Diagnosis/ histology …….
Histology number…….
Date of Diagnosis…….
B symptoms…. Present……. Absent……Not available…….
Bone marrow…Not involved……Involved……Not available……
Bulky disease…Absent……Present……Not available……
Ann Arbor Stage …I……. II……III……IV
ECOG…1…….2……3…….4……. not available……
Extra nodal site(s)…….
LDH Levels(units/ml) ……. not available
Albumin levels(g/L) ……not available
IPI Score…0……1……2……3……4……5…….
HIV status……Positive……. Negative……Unknown…….

Date diagnosed……

Immunohistochemistry

CD 20 ……
BCL6…….
BCL2…… …… CD45……
CD 3 …….
IRF4/MUM1…….
CD10 …….
Ki67%……

C. Treatment history

Treatment regimen…….
First line……from(date)……to(date)
number of cycles…….
Salvage…… from(date)……...to(date)
Number of cycles…….
Radiotherapy……
Dose…….
HAART regimen……Date started……

IPI SCORE

Age greater than 60 years

Stage III or IV disease

Elevated serum LDH

ECOG performance status of >2

More than 1 extranodal site

The sum of the points allotted correlates with the following risk groups:

Low risk (0-1 points) - 5-year survival of 73%

Low-intermediate risk (2 points) - 5-year survival of 51%

High-intermediate risk (3 points) - 5-year survival of 43%

High risk (4-5 points) - 5-year survival of 26%

Appendix II: Standard Immunohistochemistry protocol

1. Slide preparation for Immunohistochemistry

a) Wash and rinse slides in tap water in a large plastic beaker
b) Immerse slides in hydrochloric acid for 30 min (MUST be done under the fume hood and in glass beakers)
c) Rinse rapidly in distilled water

d) Immerse slides in diluted poly-L Lysine adhesive (70ml Poly-L-adhesive) DAKO and top the volume to 2 liters with PBS) for 30 min at room temperature
e) Rinse slides rapidly in distilled water
f) Air dry slides at 37°C over night
g) Pack the slides, label and store for use

2. Antigen retrieval

a) Section the chosen block at 4-5microns and mount on to the above slides
b) Incubate overnight at 37°C
c) The following day, deparaffinize in Xylene and take sections to water (slides should not be allowed to dry)
d) Drain slides and quench to block endogenous peroxidase (3% H_20_2 in TBS-Ph 7.6 for 10 min)
e) Rinse slides twice in distilled water
f) Heat induced antigen retrieval
g) Immerse the slides in plastic coplin jars filled with antigen retrieval solution (Citrate buffer)
h) Insert coplin jar in the microwave and retrieve for 15min at 5 min intervals adding more distilled water in between. Retrieval time varies and should be optimized\
i) Remove coplin jar at the end of the retrieval process and let stand to cool

3. Antibody application

a) Arrange slides in a humidifier chamber, marking the area of antibody application with grease pencil
b) Rinse the slides with Tris/HCL wash buffer with Tween 20
c) Drain the slides and block non-specific binding sites using the specified serum
d) Drain slides (do not rinse the slides) and apply diluted primary antibody
e) Incubate for one and half hours at room temperature. (overnight incubation at 4°C yields better results)
f) Rinse slides with Tris/Tween 20 wash buffer
g) Incubate with secondary antibody for 30min
h) Rinse in Tis/Tween buffer
i) Incubate with tertiary antibody for 30min
j) Rinse in Tis/Tween buffer
k) Incubate with chromogenic substrate solution (DAB or diluted FUSCHIN solution in specified buffer). Length of staining should be optimized (approximately 7 min)
l) Rinse with Tris/Tween 20 buffer wash
m) Put the slides in a slowly flowing tap water, taking attention not to wash off the tissue

n) Counter stain with Mayers' hematoxylin

o) Blue the sections in Scotts tap water

p) Dehydrate the slides in xylene and ascending series of ethanol concentrations and mount

4. Preparation of buffers

Preparation of TBS

a) 60.55gm of Tris powder in 1 Liter of distilled water (de-ionized water is better). PH 7.6

NB: Use PBS as alternative (PH 7.6)

Prepation of Tween 20 TBS

a) Final concentration of Tween 20 (commercial solution) in tbs is 0.005%

Preparation of citrate buffer ph 6.0

a) Prepared from 36ml of solution A+164ml solution B (Top the total volume to 2000mls)

b) Solution A: 10.505g of citric acid in 500ml distilled water

c) Solution B: 14.705g of sodium citrate-2-hydrate ($C_6H_5Na_3O_7$ Mol. Wt. 294.10g/ml/ 500ml distilled water

Appendix III: Participant information form(adult)

Title of Study: ***The clinicopathological profile of diffuse large B cell lymphoma managed at KNH***

Principal Investigator and institutional affiliation:
Dr Oyiro O. Peter, Department of Clinical Medicine and Therapeutics, School of Medicine, The University of Nairobi
peteroyiro@gmail.com, 0700934072
Co-Investigators and institutional affiliation:

1. DR. N.A. Othieno-Abinya- MB.Ch. B, MMed, FRCP,
Medical Oncologist and Professor of Medicine,
Department of Clinical Medicine and Therapeutics, University of Nairobi
naoabinya@hurlighamoncology.co.ke

2. Prof. Lorenzo Leoncini, MD, Department of Medical Biotechnology
Director of Pathological Anatomy Division, University of Siena, Italy
leoncinil.usisi.it

3. Nyagol Joshua, PhD, Department of Human Pathology
Unit of Immunology, University of Nairobi.
josnyagol@gmail.com

Introduction: I would like to tell you about a study being conducted by the above listed researchers. The purpose of this consent form is to give you the information you will need to help you decide whether to be a participant in the study. Feel free to ask any questions about the purpose of the research, what happens if you participate in the study, the possible risks

and benefits, your rights as a volunteer, and anything else about the research or this form that is not clear. When we have answered all your questions to your satisfaction, you may decide to be in the study or not. This process is called 'informed consent'. Once you understand and agree to be in the study, I will request you to sign your name on this form. You should understand the general principles which apply to all participants in a medical research: i) Your decision to participate is entirely voluntary ii) You may withdraw from the study at any time without necessarily giving a reason for your withdrawal iii) Refusal to participate in the research will not affect the services you are entitled to in this health facility or other facilities. We will give you a copy of this form for your records. May I continue? YES / NO

This study has approval by The Kenyatta National Hospital-University of Nairobi Ethics and Research Committee Protocol No. P83/02/2018.

WHAT IS THIS STUDY ABOUT?

The researchers listed above are interviewing individuals who have a type cancer called Non-Hodgkins Lymphoma who are on treatment of follow-up at KNH. The purpose of the interview is to find out the characteristics and types of this disease that is common in our country. Participants in this research study will be asked questions about the presentation of their disease and treatment received. We will also with your permission access your file to extract information vital for this study. There will be approximately 80 participants in this study randomly chosen. We are asking for your consent to consider participating in this study.

WHAT WILL HAPPEN IF YOU DECIDE TO BE IN THIS RESEARCH STUDY?

If you agree to participate in this study, the following things will happen:

You will be interviewed by a trained interviewer in a private area where you feel comfortable answering questions. The interview will last approximately 15minutes. The interview will cover topics such as disease presentation, investigations done so far including HIV status, social background. After the interview, we will request to retrieve your diagnostic specimen and ship it to a more specialized laboratory in Siena, Italy for further analysis. We will ask for a telephone number where we can contact you if necessary. If you agree to provide your contact information, it will be used only by people working for this study and will never be shared with others. The reasons why we may need to contact you is so as we may want to relay the results of the further tests to help in your management

ARE THERE ANY RISKS, HARMS DISCOMFORTS ASSOCIATED WITH THIS STUDY? Medical research has the potential to introduce psychological, social, emotional and physical risks. Effort should always be put in place to minimize the risks. One potential risk of being in the study is loss of privacy. We will keep everything you tell us as confidential as possible. We will use a code number to identify you in a password-protected computer database and will keep all our paper records in a locked file cabinet. However, no system of protecting your confidentiality can be secure, so it is still possible that someone could find out you were in this study and could find out information about you. Also, answering questions in the interview may be uncomfortable for you. If there are any questions you do not want to answer, you can skip them. You have the right to refuse the interview or any questions asked during the interview. All study staff and interviewers are professionals with special training in these examinations/interviews.

ARE THERE ANY BENEFITS BEING IN THIS STUDY? You may benefit by receiving free further tests that help redefine your diagnosis and aid in your treatment. We will refer you to other points of care and support where necessary. Also, the information you provide will help us better understand this disease called lymphoma. This information is a contribution to science.

WILL BEING IN THIS STUDY COST YOU ANYTHING? No.
WILL YOU GET REFUND FOR ANY MONEY SPENT AS PART OF THIS STUDY? Yes, we will reimburse you for the cost incurred in transport to the facility when called upon.
WHAT IF YOU HAVE QUESTIONS IN FUTURE?
If you have further questions or concerns about participating in this study, please call or send a text message to the study staff at the number provided at the bottom of this page. For more information about your rights as a research participant you may contact the Secretary/Chairperson, Kenyatta National Hospital-University of Nairobi Ethics and Research Committee Telephone No. 2726300 Ext. 44102 email uonknh_erc@uonbi.ac.ke. The study staff will pay you back for your charges to these numbers if the call is for study-related communication.
WHAT ARE YOUR OTHER CHOICES?
Your decision to participate in research is voluntary. You are free to decline participation in the study and you can withdraw from the study at any time without injustice or loss of any benefits.

Appendix IV: Adult consent form (statement of consent)

participant's statement:
I have read this consent form or had the information read to me. I have had the chance to discuss this research study with a study counselor. I have had my questions answered in a language that I understand. The risks and benefits have been explained to me. I understand that my participation in this study is voluntary and that I may choose to withdraw any time. I freely agree to participate in this research study. I understand that all efforts will be made to keep information regarding my personal identity confidential
By signing this consent form, I have not given up any of the legal rights that I have as a participant in a research study. I agree to participate in this research study: Yes, No I agree to have (biopsy tissue paraffin embedded block and or slides) preserved for later study:
Yes, No I agree to provide contact information for follow-up:
Participant name: _____
Participant signature Thumb stamp _____ Date _____
Researcher's statement I, the undersigned, have fully explained the relevant details of this research study to the participant named above and believe that the participant has understood and has willingly and freely given his/her consent. Researcher 's Name: _____ Date: _____
Signature_____
Role in the study: Principal Investigator.
For more information contact:
Principal Investigator Dr Oyiro Peter at 0700934072/0711844366 from 8am to 5pm
Supervisor Prof Abinya 0722809030, naoabinya@hurlinghamoncology.co.ke

Appendix V: Maelezo ya idhini (Mtu mzima)

Kichwa cha Utafiti: *The clinicopathological profile of diffuse large B cell lymphoma* managed *at Kenyatta National Hospital*
Mtafiti Mkuu na ushirikiano wa taasisi: Dr Oyiro Peter, Idara ya Dawa ya Kliniki na Matibabu, Shule ya Matibabu, Chuo Kikuu cha Nairobi
Wachunguzi wa ushirikiano na ushirika wa taasisi:
1. DR. N.A. Othieno-Abinya- MB.Ch. B, M'med, FRCP,
Medical Oncologist and Professor of Medicine,
Department of Clinical Medicine and Therapeutics, University of Nairobi

2. Prof. Lorenzo Leoncini, MD, Department of Medical Biotechnology
Director of Pathological Anatomy Division, University of Siena, Italy

3. Nyagol Joshua, PhD, Department of Human Pathology
Unit of Immunology, University of Nairobi.

Utangulizi: Jina langu ni Dkt Oyiro Peter, mwanafunzi katika kitengo Clinical Medicine and Therapeutics, School of Medicine, chuo kikuu cha Nairobi. Ninafanya utafiti kuhusu saratani ya Lymphoma. Utafiti huu utatusaidia kuchunguza huu ugonjwa ili tuweze kuufahamu vizuri Zaidi na kutuwezesha kuwahudumia hawa kwa hali ya juu zaidi.Wewe/mtoto wako mumechaguliwa sababu mnao huu ugonjwa. Utafiti utafanywa katika chuo kikuu cha Nairobi, idara ya Human Pathology.
Ningependa kukuambia kuhusu utafiti uliofanywa na watafiti waliotajwa hapo juu.

Madhumuni ya fomu hii ya idhini ni kukupa taarifa unayohitaji ili kukusaidia uamuzi au ikiwa ni mshiriki katika utafiti. Jisikie huru kuuliza maswali yoyote kuhusu madhumuni ya utafiti, kinachotokea ikiwa unashiriki katika utafiti, hatari na faida iwezekanavyo, haki zako kama kujitolea, na kitu kingine chochote kuhusu utafiti au fomu hii ambayo haijulikani. Tunapojibu maswali yako yote kwa kuridhika kwako, unaweza kuamua kuwa katika utafiti au la. Utaratibu huu unaitwa 'kibali cha habari'. Mara unapoelewa na kukubali kuwa katika utafiti, nitawaomba usaini jina lako kwenye fomu hii. Unapaswa kuelewa kanuni za jumla ambazo zinatumika kwa washiriki wote katika utafiti wa matibabu:

i) Uamuzi wako wa kushiriki ni kikamilifu kwa hiari

ii) Unaweza kujiondoa kwenye utafiti wakati wowote bila ya kutoa sababu ya uondoaji wako

iii) Kukataa kushiriki katika utafiti hauathiri huduma unazostahili kwenye kituo hiki cha afya au vifaa vingine. Tutakupa nakala ya fomu hii kwa rekodi zako.

Naweza kuendelea? NDIYO / Hapana

Utafiti huu una kibali na Chuo Kikuu cha Taifa cha Kenyatta-Chuo Kikuu cha Nairobi Kitivo cha Maadili na Utafiti No. No. P83/02/2018

NI NINI KUJIFUNZA KUFANYA?

Watafiti waliotajwa hapo juu ni kuhojiana na watu ambao wako na ugonjwa wa saratani ya lymphoma. Kusudi la mahojiano ni kuchunguza huu ugonjwa ili tuweze kuufahamu vizuri zaidi na kutuwezesha kuwahudumia hawa kwa hali ya juu zaidi.Wewe/mtoto wako mumechaguliwa sababu mnao huu ugonjwa. Utafiti utafanywa katika chuo kikuu cha Nairobi, idara ya Human Pathology.

Washiriki katika utafiti huu wa utafiti wataulizwa maswali kuhusu dalili ya ugonjwa. Kutakuwa na washiriki wapatao 80 katika utafiti huu kwa nasibu waliochaguliwa. Tunaomba ridhaa yako kufikiria kushiriki katika utafiti huu.

NINI ITAFANYIKA UKIKUBALI KUSHIRIRKI KATIKA UTAFITI HUU?

Ikiwa unakubali kushiriki katika somo hili, mambo yafuatayo yatatokea:

Utaulizwa na mhojiwaji mwenye ujuzi katika eneo la kibinafsi ambako unasikia kujibu maswali. Mahojiano itaendelea takriban 15 minutes. Mahojiano yatashughulikia mada kama vile uwasilishaji wa magonjwa, uchunguzi uliofanywa hadi sasa unahusisha hali ya ugonjwa na historia ya kijamii. Baada ya mahojiano imekamilisha, tutaomba kutuma specimen yako ya uchunguzi kwa uchambuzi zaidi nje ya nchi katika maabara makuu huko Siena, Italy. Tutaomba namba ya simu ambapo tunaweza kuwasiliana na wewe ikiwa ni lazima. Ikiwa unakubaliana kutoa maelezo yako ya mawasiliano, itatumiwa tu na watu wanaofanya kazi kwa ajili ya utafiti huu na kamwe hawatashirikiwa na wengine. Sababu ambazo tunaweza kuwasiliana na wewe ni kama tunavyoweza kuitaka matokeo ya vipimo vya ziada ili kusaidia katika usimamizi wako

Je, kuna baadhi ya maadili, magonjwa yanayotokana na mafunzo haya? Utafiti wa matibabu una uwezo wa kuanzisha hatari za kisaikolojia, kijamii, kihisia na kimwili. Jitihada zinapaswa kuwekwa daima ili kupunguza hatari. Hatari moja ya kuwa katika utafiti ni kupoteza faragha. Tutaweka kila kitu unachotuambia kama siri iwezekanavyo. Tutatumia nambari ya nambari ili kukutambua kwenye databana la kompyuta iliyohifadhiwa na nenosiri na kuhifadhi kumbukumbu zote za karatasi kwenye baraza la mawaziri lililofungwa. Hata hivyo, hakuna mfumo wa kulinda siri yako inaweza kuwa salama kabisa, kwa hivyo bado inawezekana kwamba mtu anaweza kujua wewe ulikuwa katika utafiti huu na anaweza kupata habari kuhusu wewe. Pia, kujibu maswali katika mahojiano inaweza kuwa na wasiwasi kwako. Ikiwa kuna maswali yoyote unayotaka kujibu, unaweza kuruka. Una haki ya kukataa mahojiano au maswali yoyote yaliyoulizwa wakati wa mahojiano. Tutafanya kila kitu tunaweza kuhakikisha kuwa hii imefanywa kwa faragha. Zaidi ya hayo, wafanyakazi wote wa utafiti na wahojiwa ni wataalamu wenye mafunzo maalum katika mitihani / mahojiano haya.

Je, kuna faida yoyote kuwa katika kujifunza hii?
Unaweza kufaidika kwa kupokea vipimo vya bure vilivyosaidia kusaidiana na utambuzi wako katika matibabu yako. Tutakuelezea kwenye vitu vingine vya huduma na msaada ikiwa inahitajika. Pia, maelezo ambayo hutoa yatatusaidia kuelewa vizuri ugonjwa huu unaoitwa lymophoma. Habari hii ni mchango kwa sayansi.
KUNA GHARAMA YOYOTE KWAKO KWA KUSHIRIRKI KATIKA UTAFITI HUU?
Hapana.
Je, utapata rejea kwa kila kitu cha kifedha kama sehemu ya kujifunza hii?
Ndio tutakulipa kwa gharama zilizosafirishwa kwa usafiri hadi kituo hicho kinapoitwa juu.
IKIWA UNGEPENDA KUULIZA MASWALI BAADAYE?
Ikiwa una maswali zaidi au wasiwasi juu ya kushiriki katika somo hili, tafadhali piga simu au tuma ujumbe wa maandishi kwa wafanyakazi wa kujifunza kwa idadi iliyotolewa chini ya ukurasa huu. Kwa habari zaidi juu ya haki zako kama mshiriki wa utafiti unaweza kuwasiliana na Katibu / Mwenyekiti, Kenyatta National Hospital-Chuo Kikuu cha Nairobi Maadili na Utafiti Kamati Namba Namba 2726300 Ext. 44102 barua pepe uonknh_erc@uonbi.ac.ke.
Wafanyakazi wa kujifunza watawalipa malipo yako kwa idadi hizi ikiwa wito ni kwa ajili ya mawasiliano inayohusiana na utafiti.
Uamuzi wako wa kushiriki katika utafiti ni wa hiari. Wewe ni huru kupungua kushiriki katika utafiti na unaweza kujiondoa kwenye utafiti wakati wowote bila udhalimu au kupoteza faida yoyote.

Appendix VI: idhini ya mtu mzima

Taarifa ya mshiriki nimesoma fomu hii ya idhini au nilisoma maelezo. Nimekuwa na fursa ya kujadili utafiti huu wa utafiti na mshauri wa utafiti. Nimekuwa na maswali yangu akajibu kwa lugha ambayo ninayoelewa. Hatari na faida zimeelezewa kwangu. Ninaelewa kuwa ushiriki wangu katika utafiti huu ni hiari na kwamba nipate kuchagua kuchagua wakati wowote. Ninakubali kwa hiari kushiriki katika utafiti huu wa utafiti. Ninaelewa kwamba jitihada zote zitafanywa ili kuweka taarifa kuhusu utambulisho wangu binafsi kwa kusaini fomu hii ya kibali, sijaacha haki yoyote ya kisheria ambayo mimi nishiriki katika utafiti wa utafiti.
Nakubali kushiriki katika utafiti huu wa utafiti: Ndiyo Hapana
_____Saini ya Washiriki / Thumb _____Tarehe _____

Taarifa ya Mtafiti
Mimi, aliyeandikwa chini, ameeleza kikamilifu maelezo ya utafiti wa utafiti huu kwa mshiriki aliyechaguliwa hapo juu na kuamini kwamba mshiriki ameelewa na ametoa kibali chake kwa hiari na kwa hiari.
Jina la Mtafiti: _____ Tarehe: _____
Saini _____Jukumu katika utafiti: _____.
wasomaji ambao walielezea fomu ya ruhusa ya habari. Kwa habari zaidi wasiliana _____ kwa _____ kutoka _____ hadi _____

Mtafiti: Daktari Oyiro Peter at 0700934072/0711844366 from 8am to 5pm
Mtafiti: Prof Abinya 0722809030, naoabinya@hurlinghamoncology.co.ke

Appendix VII: Participant information and consent form Parental consent

Title of Study: The clinicopathological profile of diffuse large B cell lymphoma managed at KNH

Principal Investigator and institutional affiliation: Dr Oyiro O. Peter, Department of Clinical Medicine and Therapeutics, School of Medicine, The University of Nairobi

Co1. DR. N.A. Othieno-Abinya- MB.Ch. B, MMed, FRCP,

Medical Oncologist and Professor of Medicine,

Department of Clinical Medicine and Therapeutics, University of Nairobi

2. Prof. Lorenzo Leoncini, MD, Department of Medical Biotechnology

Director of Pathological Anatomy Division, University of Siena, Italy

3. Nyagol Joshua, PhD, Department of Human Pathology

Unit of Immunology, University of Nairobi.

Introduction:

I would like to tell you about a study being conducted by the above listed researchers. The purpose of this consent form is to give you the information you will need to help you decide whether your child should participate in the study. Feel free to ask any questions about the purpose of the research, what happens if your child participates in the study, the possible risks and benefits, the rights of your child as a volunteer, and anything else about the research or this form that is not clear. When we have answered all your questions to your satisfaction, you may decide if you want your child to be in the study or not. This process is called 'informed consent'. Once you understand and agree for your child to be in the study, I will

request you to sign your name on this form. You should understand the general principles which apply to all participants in a medical research:

i) Your child decision to participate is entirely voluntary

ii) You child may withdraw from the study at any time without necessarily giving a reason for his/her withdrawal

iii) Refusal to participate in the research will not affect the services your child is entitled to in this health facility or other facilities.

May I continue? YES / NO

For children below 18 years of age we give information about the study to parents or guardians. We will go over this information with you and you need to give permission for your child to participate in this study. We will give you a copy of this form for your records.
Add comment on ascent (e.g. If the child is at an age that he/she can appreciate what is being done the he/she will also be required to agree to participate in the study after being fully informed).

WHAT IS THE PURPOSE OF THE STUDY?

The researchers listed above are interviewing individuals who have a type cancer called Non-Hodgkins Lymphoma who are on treatment of follow-up at KNH. The purpose of the interview is to find out the characteristics and types of this disease that is common in our country. Participants in this research study will be asked questions about the presentation of their disease and treatment received. We will also with your permission access your file to extract information vital for this study. There will be approximately 80 participants in this study randomly chosen. We are asking for your consent to consider participating in this study.

WHAT WILL HAPPEN IF YOU DECIDE YOU WANT YOUR CHILD TO BE IN THIS RESEARCH STUDY?

If you agree for your child to participate in this study, the following things will happen:

You will be interviewed by a trained interviewer in a private area where you feel comfortable answering questions. The interview will last approximately 15minutes. The interview will cover topics such as disease presentation, investigations done so far including HIV status, social background. After the interview has finished, we will request to retrieve your child's diagnostic specimen and ship it to a more specialized laboratory in Siena, Italy for further analysis. We will ask for a telephone number where we can contact you if necessary. If you agree to provide your contact information, it will be used only by people working for this study and will never be shared with others. The reasons why we may need to contact you is so as we may want to relay the results of the further tests to help in your child's management.

ARE THERE ANY RISKS, HARMS, DISCOMFORTS ASSOCIATED WITH THIS STUDY?

Medical research has the potential to introduce psychological, social, emotional and physical risks. Effort should always be put in place to minimize the risks. One potential risk of being in the study is loss of privacy. We will keep everything you tell us as confidential as possible. We will use a code number to identify your child in a password-protected computer database and will keep all our paper records in a locked file cabinet. However, no system of protecting confidentiality can be secure so it is still possible that someone could find out your child was in this study and could find out information about your child.

Also, answering questions in the interview may be uncomfortable for you. If there are any questions you do not want to answer, you can skip them. You have the right to refuse the interview or any questions asked during the interview.

All study staff and interviewers are professionals with special training in these examinations/interviews.

ARE THERE ANY BENEFITS BEING IN THIS STUDY?

Your child may benefit by receiving free specialized further testing which may inform additional better treatment and follow-up. We will refer your child to a hospital for care and support if necessary. Also, the information you provide will help us better understand the nature of this disease to initiate better plans of management. This information is a major contribution to science

WILL BEING IN THIS STUDY COST YOU ANYTHING? NO.

Any expenses incurred for this study will be reimbursed.

IS THERE REIMBURSEMENT FOR PARTICIPATING IN THIS STUDY? YES

We will reimburse for any costs such as transport, phone call expenses if incurred specifically for the study

WHAT IF YOU HAVE QUESTIONS IN FUTURE?

If you have further questions or concerns about your child participating in this study, please call or send a text message to the study staff at the number provided at the bottom of this page.

For more information about your child's rights as a research participant you may contact the Secretary/Chairperson, Kenyatta National Hospital-University of Nairobi Ethics and Research Committee Telephone No. 2726300 Ext. 44102 email uonknh_erc@uonbi.ac.ke.

The study staff will pay you back for your charges to these numbers if the call is for study-related communication.

WHAT ARE YOUR OTHER CHOICES?

Your decision to have your child participate in this research is voluntary. You are free to decline or withdraw participation of your child in the study at any time without injustice or loss of benefits.

Just inform the study staff and the participation of your child in the study will be stopped. You do not have to give reasons for withdrawing your child if you do not wish to do so. Withdrawal of your child from the study will not affect the services your child is otherwise entitled to in this health facility or other health facilities.

For more information contact the Principal Investigator, Dr Oyiro Peter at 0700934072 at from 8am to 5pm.

Appendix VIII: Child Assent form (statement of consent)

The person being considered for this study is unable to consent for him/herself because he or she is a minor (a person less than 18 years of age). You are being asked to give your permission to include your child in this study.

Parent/guardian statement

I have read this consent form or had the information read to me. I have had the chance to discuss this research study with a study counselor. I have had my questions answered by him or her in a language that I understand. The risks and benefits have been explained to me. I understand that I will be given a copy of this consent form after signing it. I understand that my participation and that of my child in this study is voluntary and that I may choose to withdraw it any time.

I understand that all efforts will be made to keep information regarding me and my child's personal identity confidential.

By signing this consent form, I have not given up my child's legal rights as a participant in this research study.

I voluntarily agree to my child's participation in this research study:

Yes No

I agree to have my child undergo specimen retrieval for further testing abroad: Yes No

I agree to have the paraffin embedded tissue block preserved for later study: Yes No

I agree to provide contact information for follow-up: Yes No

Parent/Guardian signature/Thumb stamp: _____Date _____

Parent/Guardian printed name: _____

Researcher's statement

I, the undersigned, have fully explained the relevant details of this research study to the participant named above and believe that the participant has understood and has knowingly given his/her consent.

Printed Name:_____**Date**: _____ **Signature**: _____

Role in the study: Principal Investigator

Dr Oyiro Peter at 0700934072/0711844366 from 8am to 5pm

Supervisor Prof Abinya 0722809030, naoabinya@hurlinghamoncology.co.ke

Appendix IX: Maelezo ya idhini (Mtoto)

Title: The clinicopathological profile of diffuse large B cell lymphoma managed at KNH

Principal Investigator and institutional affiliation:
Dr Oyiro O. Peter, Department of Clinical Medicine and Therapeutics, School of Medicine, The University of Nairobi.
Wachunguzi wa ushirikiano na ushirika wa taasisi
1. DR. N.A. Othieno-Abinya- MB.Ch. B, MMed, FRCP,
Medical Oncologist and Professor of Medicine,
Department of Clinical Medicine and Therapeutics, University of Nairobi

2. Prof. Lorenzo Leoncini, MD, Department of Medical Biotechnology
Director of Pathological Anatomy Division, University of Siena, Italy

3. Nyagol Joshua, PhD, Department of Human Pathology
Unit of Immunology, University of Nairobi
Utangulizi:
Ningependa kukuambia kuhusu utafiti uliofanywa na watafiti waliotajwa hapo juu. Kusudi ya fomu hii ya idhini ni kukupa taarifa unayohitaji ili kukusaidia kuamua kama la mtoto wako anapaswa kushiriki katika utafiti. Jisikie huru kuuliza maswali yoyote kuhusu kusudi la utafiti, kinachotokea ikiwa mtoto wako anashiriki katika utafiti, hatari na faida iwezekanavyo, haki za mtoto wako kama kujitolea, na chochote kingine kuhusu utafiti au fomu hii ambayo haijulikani.
Tunapojibu maswali yako yote kwa kuridhika kwako, unaweza kuamua ikiwa unataka mtoto kuwa katika utafiti au la. Utaratibu huu unaitwa 'kibali cha habari'. Mara baada ya kuelewa na kukubaliana kwa mtoto wako kuwa katika utafiti, nitawaomba usaini jina lako kwenye fomu hii. Wewe wanapaswa kuelewa kanuni za jumla zinazotumika kwa washiriki wote katika utafiti wa matibabu:
i)Uamuzi wako wa mtoto kushiriki ni kikamilifu kwa hiari
ii) Wewe mtoto huondoka kwenye utafiti
wakati wowote bila lazima kutoa sababu ya uondoaji wake
iii) kukataa kushiriki katika utafiti hauathiri huduma zako mtoto ana haki katika kituo hiki cha afya au vifaa vingine.
Naweza kuendelea? NDIO LA
Kwa watoto walio chini ya umri wa miaka 18 tunatoa maelezo juu ya utafiti kwa wazazi au walezi. Sisi utaenda juu ya habari hii na wewe na unahitaji kutoa idhini ili mtoto wako awe kushiriki katika utafiti huu. Tutakupa nakala ya fomu hii kwa rekodi zako.
Ongeza maoni juu ya kuongezeka (e.g Ikiwa mtoto ana umri wa miaka ambayo anaweza kufahamu kile kinachofanyika yeye atastahili pia kukubali kushiriki katika utafiti baada ya kuwa na habari kamili).
NINI MADA YA KUFUNZA?
Watafiti waliotajwa hapo juu ni kuhojiana na watoto ambao wako na ugonjwa wa saratani ya lymphoma. Kusudi la mahojiano ni kuchunguza huu ugonjwa ili tuweze kuufahamu vizuri zaidi na kutuwezesha kuwahudumia hawa watoto kwa hali ya juu zaidi. Mtoto wako amechaguliwa sababu ana huu ugonjwa. Utafiti utafanywa katika chuo kikuu cha Nairobi, idara ya Human Pathology na pia katika maabara ya inje uko Siena, Italy. Kusudi ya mahojiano ni kuchunguza huu ugonjwa ili tuweze kuufahamu vizuri zaidi na kutuwezesha kuwahudumia hawa kwa hali ya juu zaidi. Mtoto wako mumechaguliwa sababu mnao huu ugonjwa wa lymophoma. Washiriki katika utafiti huu watakuwa themanini (80). Tunauliza

Mtoto wako anaweza kujisikia wasiwasi wakati wa mahojiano.
Je, kuna faida yoyote kuwa katika kujifunza hii?
Mtoto wako anaweza kufaidika kwa kupokea bure uchunguzi zaidi ya specimen yake. Pia maelezo unayoyatoa itatusaidia kuelewa vizuri huu ugonjwa ndio tutibu na kufuatilia wagonjwa kwa njia ya kisasa. Habari hii pia ni mchango mkubwa kwa sayansi.
KUNA GHARAMA YOYOTE KWAKO KWA KUSHIRIRKI KATIKA UTAFITI HUU? Hapana.
Je, utapata rejea kwa kila kitu cha kifedha kama sehemu ya kujifunza hii?
Ndio tutakulipa kwa gharama zilizosafirishwa kwa usafiri hadi kituo hicho kinapoitwa juu.
NINI UNA MAFUNZO KATIKA KWANZA?
Ikiwa una maswali zaidi au wasiwasi juu ya mtoto wako kushiriki katika utafiti huu, tafadhali piga simu au tuma ujumbe wa maandishi kwa wafanyakazi wa kujifunza kwa idadi iliyotolewa chini ya ukurasa huu. Kwa maelezo zaidi kuhusu haki za mtoto wako kama mshiriki wa utafiti unaweza kuwasiliana nao Katibu / Mwenyekiti, Hospitali ya Taifa ya Kenyatta-Chuo Kikuu cha Maadili na Utafiti wa Nairobi Kamati ya Namba Namba 2726300 Ext. 44102 barua pepe uonknh_erc@uonbi.ac.ke. Wafanyakazi wa kujifunza watawalipa malipo yako kwa namba hizi ikiwa wito ni kwa ajili ya kujifunza mawasiliano.
Uamuzi wako wa kuwa na mtoto wako kushiriki katika utafiti huu ni hiari. Wewe ni huru kupungua au kuondoa ushiriki wa mtoto wako katika utafiti wakati wowote bila udhalimu au kupoteza faida.
Wajulishe watumishi wa utafiti na ushiriki wa mtoto wako katika utafiti utaacha. Unafanya haipaswi kutoa sababu za kumtoa mtoto wako ikiwa hutaki kufanya hivyo. Kuondolewa kwa mtoto wako kutoka kwenye utafiti haathiri huduma zako mtoto ni nyingine
Uwezo wa hekima katika hali hii ya afya au vituo vingine vya afya.
Kwa habari zaidi wasiliana Daktari Oyiro Peter kwa 0700934072 kutoka saa mbili asubuhi hadi saa kuma na moja jioni
Mtu anayezingatiwa kwa utafiti huu hawezi kujitolea kwa sababu yeye mtu chini ya umri wa miaka 18. Unatakiwa kutoa ruhusa yako kwa mtoto wako kushiriki katika utafiti huu.
Kuelezea mzazi / mwalimu
Nimeisoma fomu hii ya kibali au nilisoma maelezo. Nimekuwa na fursa ya kujifunza juu wa huu utafiti na mshauri. Nimekuwa na maswali yangu yamejibiwa na yeye katika lugha amabayo ninaelewa. Hatari na faida zimeelezewa kwangu. Ninaelewa kwamba nitapewa nakala ya fomu hii ya idhini baada ya kusaini. Ninaelewa kwamba ushiriki wangu na mtoto wangu katika utafiti huu ni hiari na kwamba nipate kuamua kuiondoa wakati wowote. Ninaelewa kuwa jitihada zote zitafanywa kuweka taarifa kuhusu mimi na usiri wa mtoto wangu mtoto. Kwa saini fomu hii ya kibali, Sijawahi kuacha haki za kisheria za mtoto wangu kama mshiriki katika utafiti huu. Mimi nikubali kwa hiari kushiriki kwa mtoto wangu katika utafiti huu wa utafiti:
Ndiyo /Hapana Nakubali kuwa mtoto wangu ashiriki katika hii utafiti
Ndio Hapana nia ya kuchukwa specimen iliyohifadhiwa kwa Jifunze baadaye and kusafirishwa ngambo.
Ndiyo Hapana
Nakubaliana kutoa maelezo ya mawasiliano kwa ajili ya kufuatilia:
Ndiyo Hapana
Msajili wa Mzazi/Msaidizi/Mshipa wa Thumb: _____
Tarehe _____ Mzazi/Mlinzi jina la kuchapishwa: _____
Taarifa ya MtafitiI, aliyeandikwa, ameeleza kikamilifu maelezo muhimu ya utafiti huu kwa utafiti. kushirikiana hapo juu na kuamini kwamba mshiriki ameelewa na amewapa wake / herconsent kwa ujuzi

Jina la Nyaraka: _____ Tarehe: _____
Signature: _____Role katika utafiti: _____ shahidi ni muhimu) _____
Jina: _____ Tarehe; _____