Immunohistochemical and other prognostic factors in B cell non Hodgkin lymphoma patients, Kampala, Uganda

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Abstract

Background
Non Hodgkin lymphomas are the most common lymphomas in Uganda. Recent studies from developed countries have shown differences in survival for the different immunophenotypes. Such studies are lacking in Africa where diagnosis is largely dependent on morphology alone. We report immunohistochemical and other prognostic factors in B cell non Hodgkin lymphoma patients in Kampala, Uganda.

Methods
Non Hodgkin lymphoma tissue blocks from the archives of the Department of Pathology, Makerere University College of Health Sciences, Kampala, Uganda, from 1991-2000, were sub typed using haematoxylin and eosin, Giemsa as well as immunohistochemistry. Using tissue micro array, 119 biopsies were subjected to: CD3, CD5, CD10, CD20, CD23, CD30, CD38, CD79a, CD138, Bcl-6, Bcl-2, IRTA-1, MUM1/IRF4, Bcl-1/cyclin D1, TdT, ALKc, and Ki-67/Mib1. Case notes were retrieved for: disease stage, chemotherapy, courses received and retrospective follow up was done for survival.

Results
Non Hodgkin B cell lymphomas comprised of Burkitt lymphoma [BL] (95/119) diffuse large B cell lymphoma (19/119), mantle cell lymphoma (4/119) and precursor B lymphoblastic lymphoma (1/119).
For Burkitt lymphoma, good prognosis was associated with receiving chemotherapy, female gender and CD30 positivity. Only receiving chemotherapy remained significant after Cox regression analysis. Diffuse large B cell lymphomas with activated germinal centre B cell (GCB) pattern (CD10+/-, BCL-6+/-, MUM+/-, CD138+/-) had better survival (98.4 months; 95% CI 89.5 -107.3) than the others (57.3 months; 95% CI 35.5 - 79.0) p=0.027 (log rank test).

Conclusions
Activated GCB Diffuse large B cell lymphoma had a better prognosis than the others. For Burkitt lymphoma, not receiving chemotherapy carried a poor prognosis. Availability of chemotherapy in this resource limited setting is critical for survival of lymphoma patients.
Introduction

Non Hodgkin B cell lymphomas (B-NHL are heterogeneous in morphology, immunophenotype and response to therapy. Recent studies have shown differences in survival based on their molecular profile\(^1\).

In developing countries, clinically aggressive subtypes such as Burkitt and diffuse large B cell lymphoma predominate and, unfortunately, result in poor outcome\(^2\). Factors that influence survival in non Hodgkin lymphomas in resource poor settings include socio economic status, stage of disease at presentation and getting a full course of treatment. In Uganda, several studies have described clinical factors associated with outcome of Burkitt lymphoma\(^3,4\).

In the developed countries, several methods including gene profiling and immunohistochemistry have been used for predicting prognosis\(^5,6\). Using the gene expression profile of germinal centre B and activated B cells, DLBCL was subdivided into 3 prognostic groups. However there are several drawbacks of gene expression profiling especially in resource constrained countries such as Uganda. It requires the use of optimally cryopreserved or fresh tissues as well as DNA micro array technology which is more costly than immunohistochemistry on paraffin sections.

Recently, several workers\(^7,8\) have used germinal centre and activated B cell immunohistochemical markers on paraffin embedded tissue blocks to classify DLBCL into three prognostic groups. These include: (a) activated non GCB (CD10-, Bcl-6-, MUM1/IRF4±, CD138+); (b) activated GCB (CD10+, Bcl-6+, MUM1/IRF4±, CD138+); and (c) non activated GCB (CD10+, Bcl-6+, MUM1/IRF4-, CD138-). They showed that patients with a germinal centre B cell profile have a much better prognosis than those with the activated B cell type. Such studies have hitherto not been carried out in Uganda. We report immunohistochemical and other prognostic factors in B cell non Hodgkin lymphoma patients in Kampala, Uganda.
Methods

Study design and sampling

A cross sectional descriptive design was used for lymphoma diagnosis and immunophenotyping, while a retrospective cohort was used to determine survival. For the cross sectional study, haematoxylin and eosin and Giemsa staining was carried out in the Department of Pathology, Makerere University and immunohistochemistry in the Unit of Hematopathology, Institute of Hematology and Clinical Oncology “L. & A. Seràgnoli”, Bologna University School of Medicine, Bologna, Italy. One hundred and twenty nine patients’ biopsies diagnosed between 1991-2000 as non Hodgkin lymphoma were sub typed using tissue microarray (TMA) and immunohistochemistry with CD3, CD5, CD10, CD20, CD23, CD30, CD38, CD79a, CD138, Bcl-6, Bcl-2, IRTA-1, MUM1/IRF4, Bcl-1/cyclin D1, TdT, ALKc, and Ki-67/Mib1.

For the retrospective cohort study we retrieved patients’ case notes from the Uganda Cancer Institute in order to obtain details of the patients disease stage, type of chemotherapy, number of courses received, whether dead or alive, time to death. Cancer registry data was also used when the addresses of the patients fell within Kyadondo County, the area covered by the Kampala Cancer Registry. One of us (LKT) and two research assistants followed up patients whose survival status was not clear. The follow up involved tracing patients to their homes (district, sub county, parish and village) in the different regions of Uganda.

The patients had been treated at the Uganda Cancer Institute which is the oldest unit for cancer treatment in the country. It began as a centre for the treatment of Burkitt lymphoma patients in the 1960s and has two units: the solid tumor treatment unit and the lymphoma treatment centre. Those with Burkitt lymphoma received COM⁹ (cyclophosphamide, vincristine, intrathecal methotrexate) whereas those with other non Hodgkin lymphomas received CHOP (cyclophosphamide, adriamycin, vincristine and prednisolone).
**Tissue micro array construction (TMA)**

Haematoxylin and eosin (H&E) stained slides were used to identify the representative tumor fields that were marked and correspondingly identified on the tissue blocks, as described in our previous paper.\(^2\) Basically, “tissue cylinders of diameter of 1mm were punched from the marked areas of each block and incorporated into a recipient paraffin block using a precision instrument, the tissue arrayer (Beecher Instruments, Silver Spring, Maryland, USA). For adequate sampling each specimen was represented in duplicate using 1mm cores in the recipient block. Three TMA recipient blocks were made; two of these had 48 punches each of Burkitt lymphoma while the other one had 33 punches of other non Hodgkin lymphomas\(^2\).

**Immunohistochemistry**

This was described in detailed in our previous publication that used the same tissue blocks and TMAs\(^2\). Basically “four-µm thick sections were cut from TMAs, coated on electrically charged slides, re-hydrated, and submitted to antigen retrieval in ethylene diamine tetra acetic acid (EDTA) 1mM (pH 8.0) by micro-waving twice for 5 minutes at either 750 or 900 W, that proved to be very efficient also in over-fixed material according to previous experience\(^10\). After cooling, the slides were put on a TechMate 500 immunostainer and incubated for 30 minutes at room temperature with antibodies against CD3, CD5, CD10, CD20, CD23, CD30, CD38, CD79a, CD138, Bcl-6, Bcl-2, IRTA-1, MUM1/IRF4, Bcl-1/cyclin D1, TdT, ALKc, and Ki-67/Mib1. Details on the antibodies, sources, dilutions and antigen retrieval are listed in Table 1\(^2\). The antibodies were detected by either the alkaline phosphatase anti-alkaline phosphatase immunocomplexes (APAAP) technique or the Envision\(^+\) technique\(^11\).

**Data management, analysis and statistical issues:**

For the descriptive cross sectional study, a sample size of 112 was calculated using a formula by Kish and Leslie\(^12\). In this calculation we assumed that the prevalence of B cell non Hodgkin’s lymphoma in the total population of non-Hodgkin’s lymphoma was 92.1% according to a Ugandan study\(^2\) with a precision of 5% and 95% confidence interval.

For the retrospective cohort design, a sample size of 52 was calculated using a formula by Fleiss with 80% power and 95% confidence interval.\(^12\) We assumed the expected
outcome among patients with NHL of germinal centre origin would be 86% and the expected outcome among patients with NHL of non germinal centre origin would be 63%.

Data were collected and entered into the computer using EPI INFO software (supplied by CDC and WHO) for storage and initial analysis. Further analysis was done using SPSS software. For continuous variables, the relevant measures of central tendency (means for normally distributed data and medians and inter-quartile ranges for skewed data) were used to explore the data. Kaplan-Meier curves and the log rank test were used to determine survival. To determine factors associated with overall survival, univariate and multivariate Cox hazards regression analysis was carried out. A p value of less than 0.05 was considered significant.

Ethical issues: Permission to carry out the study was obtained from the Makerere University Faculty of Medicine Research and Ethics Committee.

Study limitations

This was a retrospective rather than prospective study making it difficult to get good socio-demographic information. Clinical outcome predictors such as the International prognostic Index were not complete. Lactate dehydrogenase was not routinely done in the patients.
Results

1. Background characteristics
The median age was 9.0 (inter-quartile range 6-15.5) years. The youngest patient was 2 years and the oldest was 64 years. The mean age was 15.7 (SD 15.5) years.

2. Diagnosis
As expected, 79.83% of the patients had Burkitt lymphoma, followed by diffuse large B cell lymphoma (15.97%), mantle cell lymphoma (3.36%), and precursor B lymphoblastic lymphoma (0.84%).

3. Results of individual immunohistochemical markers
B cell non Hodgkin lymphoma immunophenotypes showed 100% CD20 positivity. BL had a consistent immunophenotype (CD10+, BCL-6+, BCL-2-, CD20+). (Table 2)

4. Burkitt lymphoma survival by treatment
Overall survival of Burkitt patients was 12.7 months (95%CI 6.0-19.4). Those who received chemotherapy had a mean survival of 18.1 months (95% CI 9.4-26.8) while those who did not receive chemotherapy had a mean survival of 0.5 months (95% CI 0.3 - 0.7). p = 0.001.

5. Burkitt lymphoma survival by CD30
The CD30 negative patients had a mean survival of 6.8 (95% CI 0.0 - 14.7) while the CD30 positive had a mean survival of 12.3 (95% CI 7.3 - 17.3), p = 0.0169.

6. Burkitt lymphoma survival by gender
The male patients had a mean survival of 3.3 (95% CI 0.81-5.8) months while the females had a mean survival of 17.7 (95% CI 8.3-27.0) months, p=0.028.
Only receiving chemotherapy remained significant after Cox regression analysis. (Table 3)
7. Diffuse large B cell lymphoma survival
Diffuse large B cell lymphomas with activated germinal centre B cell (GCB) pattern (CD10+/−, BCL-6+/−, MUM+/−, CD138+/-) had better survival (98.4 months; 95% CI 89.5 - 107.3) than the others (57.3 months; 95% CI 35.5 - 79.0) p=0.027 (log rank test). (Figure 1)

Discussion
The aim of this paper was to classify B cell non Hodgkin lymphomas in Uganda using immunohistochemical markers and correlate this to patient outcome.

In the current study, B cell non Hodgkin lymphomas seem to be affecting mainly young people, median age of 9 years. The mean age was 15.7 years consistent with results of previous similar studies from Uganda2, but much lower than that reported from Kenya by Cool and Bitter.13

Overall survival of patients with B cell non Hodgkin lymphomas
Burkitt lymphoma
The overall survival of patients with B cell non Hodgkin lymphomas was 60 months but only 12.7 months in patients with Burkitt lymphoma. This survival of patients with Burkitt lymphoma in this series is surprisingly much lower than what was reported from the same centre in the 1970s/1980s by Olweny and others.14 This difference could possibly be due to the fact that Olweny’s studies were done on a cohort that was meticulously followed up, unlike the current investigation in which we studied routine patients who came to the Uganda Cancer Institute for treatment and were not particularly followed up or sought after to complete treatment.

In the current study factors associated with survival included receiving chemotherapy, female gender and CD30 positivity. As expected those who did not receive chemotherapy died within the first month after presentation to hospital. The possible reason for this is that the Uganda Cancer institute is grossly under funded and therefore patients have to buy anticancer drugs from private pharmacies. Unfortunately, most patients are poor and therefore cannot afford the drugs15. Many patients are referred from rural areas to the main cancer treatment centre in Kampala at the Uganda Cancer Institute and this constitutes further delay.
In Uganda, the standard regimen for treating Burkitt lymphoma is cyclophosphamide, vincristine, prednisolone (COM) and intrathecal methotrexate for central nervous system disease prophylaxis. In our study, those who received chemotherapy with COM had a better overall survival than those who did not. Endemic BL is characterised by a very high proliferative index nearing 100%, and the disease is very rapidly progressive with a doubling time of 24 hours and is fatal if not treated early with intensive chemotherapy regimens.

Another factor associated with poor survival was male gender. Similar observations have been made by other authors in studies on childhood cancers. The reasons for the difference in prognosis between males and females among patients with Burkitt lymphoma are not very clear. However one explanation is that males have an inherent tendency to have higher rates of cell division than females. The fact that the growth rate of the male embryo is higher than that of the female has been suggested as lending credence to this hypothesis.

Of interest in our study is the fact that CD30 positive Burkitt lymphoma patients had a better survival than those who were CD30 negative. No prognostic significance has been previously found in Burkitt lymphoma regarding whether they are positive or negative for CD30. We have previously reported a high incidence of CD30 in Burkitt lymphoma patients in Uganda.

Survival of patients with DLBCL
In our study, diffuse large B cell lymphomas fell into three distinct groups with independent prognostic significance. These included: (a) non activated GCB, (b) activated GCB, and (c) activated non GCB. These are similar to the groups identified by researchers in the developed countries.

Whereas studies in the developed countries have found that non activated GCB had the best prognosis, in our study we have found that patients with activated germinal centre B-cell lymphoma (type B) had the best prognosis. The reasons for this difference are not clear and given the small numbers will have to be confirmed by larger studies. The
difference could also be a reflection of yet unrecognized molecular heterogeneity in the tumors\textsuperscript{23}.

The overall survival of the patients with DLBCL in our study was lower than that reported from the developed countries. This could be related to the fact that in Uganda patients with DLBCL are treated with cyclophosphamide, adriamycin, vincristine, prednisolone (CHOP) rather than the more effective cyclophosphamide, adriamycin, vincristine, prednisolone plus Rituximab (CHOP-R) that has become standard of care in the developed countries\textsuperscript{24}.

**Conclusion**

Immunohistochemistry on paraffin embedded tissue blocks using selected GCB and activation markers has yielded important information which predicts the outcome of patients with non Hodgkin lymphomas in Uganda. Generally the Ugandan patients studied had a very poor prognosis. A number of factors including lack of timely chemotherapy seem to be responsible for this. Availability of chemotherapy in this resource limited setting is critical for survival of lymphoma patients.

**Contribution of the authors:**

LKT, SAP and WB conceptualised the study. LKT, CA, CC, SAP and SR carried out the immunohistochemistry. EO, HW provided clinical information and tissue blocks, WB and SAP supervised the study. LKT analysed the data and wrote the manuscript. SAP revised the manuscript. PP and BF provided some primary antibodies and revised the manuscript. All authors have read through and approved the manuscript.

**Conflict of interest:**
All authors have no conflict of interest to declare

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References:


Additional files provided with this submission:

Additional file 1: tables 1 2 3.doc, 54K
http://www.biomedcentral.com/imedia/3529552292914535/supp1.doc
Additional file 2: b cell nhl in uganda.pdf, 2195K
http://www.biomedcentral.com/imedia/1215280762951236/supp2.pdf