

Incidence of and factors associated with discordant pathologic diagnosis in patients with suspected Burkitt Lymphoma treated in a low-resource setting

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Abstract

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Aim: To determine the frequency of discordant diagnosis of endemic Burkitt Lymphoma at the Uganda Cancer institute and to evaluate different baseline patient characteristics for their association with discordance and its impact on one year overall survival

Background: Burkitt Lymphoma is the most common childhood malignancy in many countries of Sub-Saharan Africa. Overall survival rates for this disease in low-resource settings are far below those seen for a similar disease in more affluent countries. Resources for pathology are scarce in many countries where Burkitt Lymphoma is endemic, leading to high rates of inaccurate diagnoses, thereby possibly contributing to the lower overall survival rates. A clinical care project at the Uganda Cancer Institute (UCI) in Kampala, Uganda has been following patients with Burkitt Lymphoma for more than 2 years, with preliminary data showing a high rate of diagnostic discordance.

Method: All patients presenting for care at the UCI between July 2012 and July 2014 with suspected BL and two independent histopathologic evaluations of the same tumor biopsy were eligible for this study. Baseline demographic and clinical characteristics were described for the entire cohort and by discordance status.

Kappa statistics were calculated to determine the rate of agreement between two pathology labs involved in the project. Poisson regression analysis was used to determine associations of baseline factors with discordance. To determine one year overall survival the Kaplan-Meier Method was used.

Findings: 57 patients were eligible for the study. The percentage of discordance for BL vs. “other” was 32%. Older age and central nervous system involvement were associated with a higher frequency of discordant diagnosis. There was a trend towards elevated LDH being associated with discordance, though this was not statistically significant. These associations were attenuated when adjusting for age. Overall survival was 55% for the entire cohort, and 70% and 39% for patients without and with discordance respectively. The difference in survival between the groups did not meet statistical significance.

Conclusions: Discordance was a frequent event in our study setting. Baseline factors associated with discordance may be used to guide decisions regarding more extensive diagnostic workup, but need to be confirmed in larger studies. Cheaper and sustainable pathology testing needs to be made available for countries with limited resources.

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List of Acronyms and Abbreviations

BL - Burkitt Lymphoma

eBL – endemic Burkitt Lymphoma

CNS – Central Nervous System

CSF – Cerebrospinal Fluid

ES – Ewings Sarcoma

FFPE – Formalin Fixed and Paraffin embedded

FISH – Fluorescent in situ hybridization

H&E – Hematoxylin and Eosin

IHC – Immunohistochemistry

LL – Lymphoblastic Lymphoma

NHL – Non-Hodgkin Lymphoma

OS – Overall survival

PNET – Peripheral Neuroectodermal Tumor

RMS – Rhabdomyosarcoma

UCI – Uganda Cancer Institute

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1. Introduction

Endemic Burkitt Lymphoma (eBL) is the most common childhood cancer in many countries of sub-Saharan Africa, with 5-15 cases per 100,000 person-years in children depending on the region. This makes it more common than acute lymphoblastic leukemia, the most common childhood cancer in western countries.[1-5] Overall survival (OS) at one year in children with eBL treated in low- and middle-income countries (LMIC) remains low (OS 40-60%),[6-10] and is significantly lower compared to OS in children with the sporadic form of the disease, mostly seen in higher-resource settings (OS~ 85%).[11-13]

There are many factors that may contribute to this discrepancy in survival. Due to lack of supportive care and drug shortages physicians find themselves unable to treat children with this disease with the same intensive treatment regimens that children with the sporadic form of the disease receive in more affluent countries. These intense regimens require a complex supportive care system, including the ability to give blood products and antibiotics. Likewise, delays in referrals to treatment facilities lead to patients presenting at late stages of the disease, when it is more difficult to achieve a good treatment response and treatment complications are more common. Another likely important contributor to poor outcomes is lack of diagnostic accuracy.[14-16] Accurate and timely pathologic diagnosis is one of the most important prerequisites for initiating appropriate tumor directed therapy. Factors associated with inaccurate diagnosis and its impact on OS have not

previously been studied in a low-resource setting, in part because of the inability to reliably track patients over time.

The diagnosis of childhood tumors relies on different staining methods. The most basic method is Hematoxylin and Eosin (H&E) staining. Burkitt Lymphoma tumor cells are classified as small round blue cell tumors, as they stain blue and are small with H&E staining. However many other childhood tumors, like rhabdomyosarcoma (RMS), primitive neuroectodermal tumor (PNET) and neuroblastoma also fall under the same category. The classic appearance of eBL on H&E staining is a “starry-sky” pattern, created, not by the tumor cells themselves, but by macrophages engulfing dead tumor cells (tingible macrophages – Figure 1 A&B). However other childhood tumors can have a similar appearance. Likewise the quality of the tumor tissue preparation needs to be of high quality for a pathologist to be able to recognize this classic pattern, a prerequisite that is not always given in settings of low resources.[12] A more accurate diagnosis is possible when performing Immunohistochemistry staining (IHC) to detect specific cell surface marker on tumor cells (for example CD20 and CD10 – Figure 1. C&D), however this is more costly and also takes specialized training to be able to interpret correctly. Therefore IHC is oftentimes not available in the setting of low-resources.

All BL tumors have a translocation involving the c-myc gene, moving this gene most commonly under the control of the heavy chain region. The translocation is detectable by Immunohistochemistry (IHC) (Figure 1. E) Staining and Fluorescent

in-situ hybridization (FISH). The cost of these tests is oftentimes prohibitive in limited resource settings, in particular if they are not performed on a regular basis.

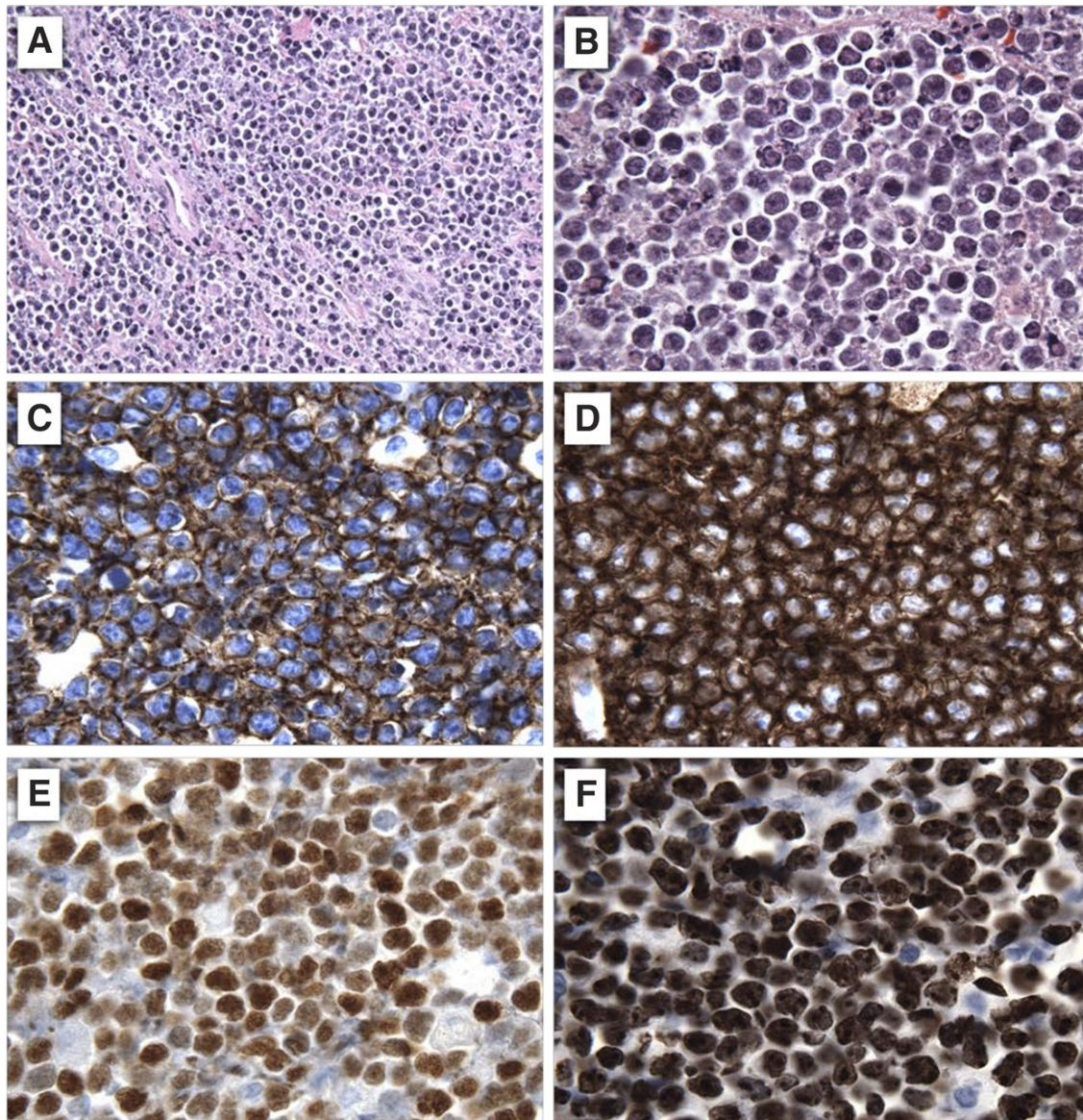


Figure 1. Histopathology of BL. Low-power (A) and high-power (B) field hematoxylin and eosin stain; CD20 (C), CD10 (D), MYC (E), and Ki67 (F) immunostains. **Caron Jacobson, and Ann LaCasce Blood 2014;124:2913-2920**

Since July 2012 the Burkitt Lymphoma (BL) project at the Uganda Cancer Institute (UCI) in Kampala, Uganda, has systematically captured data on children presenting

to this institution with suspected eBL. The pathology lab associated with the UCI to which biopsy samples were routinely sent, had long turn-around times, of up to four weeks, making the diagnosis of a fast-growing tumor like BL challenging. To improve turn-around times for pathologic diagnoses tumor biopsies of patients with suspected BL are sent to two different pathology laboratories, one of which is a local private lab with shorter processing times. Both labs formalin fix and embed the tumor biopsies in paraffin blocks (FFPE blocks) and then perform H&E staining, followed by review by a pathologist. Due to cost constraints IHC is not routinely performed. In close to 60 of the patients, two different pathology labs were used and preliminary data show that in more than 30% of the cases the pathology labs do not agree on the final diagnosis (termed discordant). Physicians then depend on clinical findings to determine appropriate treatments. The potential for a misdiagnosis leading to patients receiving the wrong therapy in this setting is therefore high.

As of March 22nd 2015, 224 patients with suspected eBL have been enrolled in this project. Of these 192 have undergone complete diagnostic workup. 140 (73%) of these patients were thought to have eBL based on clinical and pathologic findings. The proposed study aims at determining the frequency of, and factors associated with discordant pathologic diagnoses and its effect on survival outcomes of children with suspected eBL in a low-resource setting. Results from this study will inform decision-making regarding the implementation of priority diagnostic interventions that are feasible and effective in a setting with limited resources and that can be tailored based on patient and tumor characteristics.

2. Specific research questions and hypotheses

Specific Aims:

Aim 1. To determine the incidence of discordant histopathologic diagnosis between two independent examinations of tumor biopsies among children presenting with suspected eBL in Uganda.

Hypothesis: Pathologic discordance is a frequent event in pediatric patients with suspected eBL in Uganda

Aim 2. To identify baseline factors associated with discordance of diagnosis among children presenting with suspected eBL in Uganda.

Hypothesis: Patients with specific baseline characteristics, like patients with abdominal tumors or older patients, are at higher risk of discordance than other patients.

Aim 3. To determine whether discordance in pathology results impact the overall survival (OS) of patients with eBL in a setting with limited resources.

Hypothesis: Patients with diagnostic inaccuracies within our cohort have an OS that is significantly lower compared to patients without diagnostic inaccuracies.

3. Literature review

3.1. Current state of pathology in low-income countries in Africa

There is a significant lack for pathology resources, both for training and for equipment in most countries of Sub-Saharan Africa. Oftentimes there is no political backing for investing in improved pathology services. [17] With lacking infrastructure and training for pathologist, oftentimes services offered are minimal, and specialized testing is usually not available. [17-19]

While there is a shortage of healthcare provider in general in most African countries, some argue that this disproportionately affects the pathology profession. In Tanzania, for example, there is a significant shortage of pathologist[17, 19], with only 15 pathologists (1 /2.5 million people). At best there is one tenth of the coverage seen in western countries.[19] One article argues that a possible reason may be that, pathologists are seen as the “doctors of the dead”, as many perceive that pathologists perform mostly autopsies, and diagnose patients with incurable disease like cancer. [17-19] Therefore many physicians may not choose this profession.

3.2. Impact of current pathology state on cancer diagnosis

There have been few publications on the impact of low resources for pathology services on diagnostic accuracy of cancers and even fewer on how this impacts outcomes of patients with cancer. A publication from St Mary’s Hospital, Lacor (Gulu, Uganda) from 2011 evaluating the diagnostic accuracy for Burkitt Lymphoma

of local pathology services demonstrated that local pathology procedures lacked a standardized protocol.[20] Furthermore the tissue sample blocks sometimes were lost and the quality of the tissue fixation was often suboptimal. Overall the diagnostic accuracy for BL of the evaluable blocks was 58 - 88% compared to an outside reference lab and did not add much to clinical accuracy. Another recent study from the Mulago National Referral Hospital evaluated the agreement of pathologic diagnosis between a local pathology lab and a reference pathology lab in the Netherlands. The agreement between the two labs was 36% with a calculated kappa of 0.046. [21] These two publications give the local context to our study and provide some background on the state of pathology in Uganda. How diagnostic discordance impacts the outcomes of patients with cancer, and specifically Burkitt Lymphoma has not been published to our knowledge.

4. Methods:

4.1. Study setting

We performed a retrospective chart review of patients enrolled in the Burkitt Lymphoma project at the Uganda Cancer Institute (UCI) between July 15th 2012 and July 15th 2014. Patients with suspected BL were actively recruited to the UCI from Mulago Hospital, oral surgery department. The catchment area of the BL project is however quite large (See **Figure 2**). Approximately 6-8 new patients with suspected BL are referred to the UCI each month. Patients aged 0-18 years clinically suspected to have BL and who had two pathologic diagnoses from lab A (Makerere University) and lab B (SurgPath) were eligible to be included in this analysis. Patients were met

by case managers involved in the BL project once identified as having suspected BL. No formal consenting was conducted as this project was set up as a clinical care project. Outcomes of patients with BL were captured as part of the BL project in a Salesforce® database. Loss to follow-up and treatment refusal was around 6%. This study received IRB exempt status from the Fred Hutchinson Cancer Research Center.

Tumor tissue procurement

Tissue procurement, processing and analysis was per standard of care at the Uganda Cancer Institute (UCI) for patients with suspected BL. Patients who had not undergone a biopsy prior to arriving at the UCI were either referred to the oral surgery department or general surgery department at Mulago Hospital depending on the location of their tumor. Patients with facial tumors underwent a core needle biopsy of their tumor in the oral surgery department and patients with abdominal tumors, or other non-facial tumors were referred to the Mulago general surgery department for an ultrasound-guided core needle biopsy. The UCI sends tumor specimens for pathohistologic diagnosis to Makerere University. Patients usually have to pay for these services. To improve turnaround time, and as external control, an additional specimen from the same patient is sent to a private local pathology lab (SurgPath), paid for by the BL project. In some cases other outside pathology labs also received tumor specimen, though this was not routinely done. As many patients were not able to afford the costs of the pathologic services at Makerere University, not every patient had two tissue diagnoses.

Tumor specimen were formal fixed and paraffin embedded in the pathology laboratories. They were then cut and fixed on slides, followed by Hematoxylin and Eosin (H&E) staining. Laboratory technicians performed tissue processing. The tissue diagnosis was made by review of the H&E stained tissue under the microscope by trained pathologists. There are several pathologists at Makerere University, who were all involved in the diagnostics of patients with suspected BL. At SurgPath, the slides with cut tissue samples were FedExed to the US, where a single pathologist was involved in the review and diagnosis.

4.2. Treatment regimen

The BL project is a clinical care project and patients enrolled in this project received care as per UCI standard. First-line treatment for BL at the UCI consists of six cycles of cyclophosphamide, vincristine, and methotrexate with intrathecal administration of methotrexate given every two weeks (and twice a week for patients with central nervous system involvement). After two cycles patients are assessed for response to treatment. Patients who do not respond or relapse are treated with second-line therapy, which typically consists of cyclophosphamide, (hydroxyl-) doxorubicin, vincristine, and prednisone usually for six planned cycles. Other clinical care is provided as per UCI standard.

4.3. Data collection

Patients with suspected BL referred to the UCI were met by BL project case managers and were enrolled in the project. As the BL project is a clinical care

project, no formal informed consent process was conducted. Patients' baseline clinical demographics and clinical characteristics were captured during the enrollment visit. Patient information from enrollment, through treatment and in follow up was captured in patient intake forms (see **Appendix 1**). Demographic information is obtained directly from the patients or their care givers and entered into patient forms, while medical information is either directly entered into patient forms by treating physicians or abstracted from medical charts by case manager or research coordinators. The BL project data clerk enters information from patient forms into the Salesforce® database. Enrollment into the project began on July 15th 2012. For the purposes of this analysis, the data was locked as of July 15th 2014 for enrollment and includes follow-up until March 22nd 2015. Data cleaning procedures were performed on site and included identifying missing patient forms and missing variables of interest. Case managers and research coordinators either identified the missing information for entry into the database or marked it as missing. Patient information was de-identified prior to exporting information from the database. Variables of interest were abstracted from the database into excel and imported to Stata for analysis.

4.4. Definitions:

1. Suspected eBL: patient enrolled in the BL project and worked up for possible eBL.
2. Discordant diagnosis: any diagnosis that is different between the two local pathology labs.

3. Concordant diagnosis: any diagnosis that is the same between the two local pathology labs.
4. Central nervous system (CNS) involvement: patient found to have signs or symptoms of CNS involvement by history or physical exam.

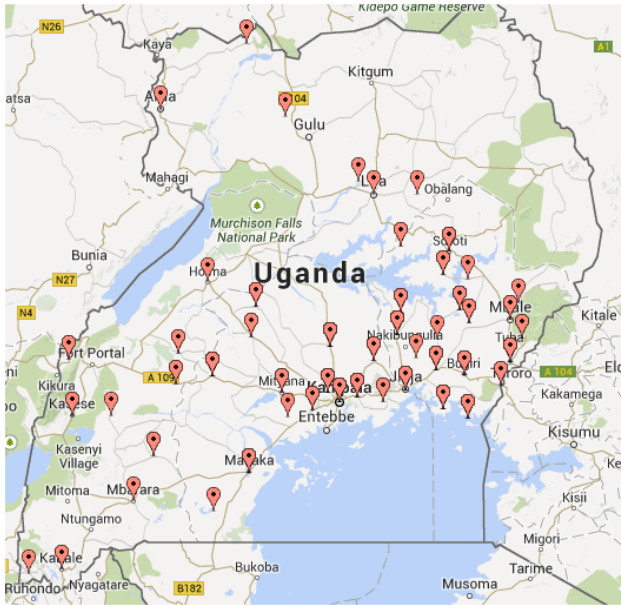


Figure 2. Catchment area of BL project (taken from BL project report 4/16/2015 courtesy Innocent Mutyaba, MD)

4.5. Statistical Methods:

Baseline demographics, clinical and pathology findings were described for the entire study cohort, as well as divided based on discordant or concordant status (Table 1). Continuous variables were represented by medians and ranges, and categorical variables by frequency counts and percentages.

For Aim 1 we computed the kappa statistic, with 95% confidence intervals (CI) to measure agreement between the 2 pathology labs. We referred to Cohen's interpretation of Kappa to determine the level of interobserver agreement (see

Appendix 2; Figure 1: Interpretation of Kappa Statistic). To test whether the 2 diagnoses are significantly different, we compared a 2-category diagnosis of eBL versus not-eBL using McNemar's test. We then identified ten categories of diagnoses to which each tissue diagnosis could be assigned, these included: Acute lymphoblastic leukemia, Burkitt Lymphoma, Hodgkin Lymphoma, Kaposi sarcoma, Lymphoblastic lymphoma, Other, Other benign, Other malignant, peripheral neuroectodermal tumor, Retinoblastoma, Rhabdomyosarcoma (see Table 5 for more details of diagnoses that were included in "Other", "Other benign" and "Other malignant"). We compared BL vs. the other nine diagnoses categories using the Bowker's test for table symmetry and the Stuart-Maxwell test for marginal homogeneity.

Preliminary data suggested that that discordance would be a frequent event affecting >10% of all patients with two diagnoses. Therefore we used Poisson regression analysis to identify baseline factors associated with discordance (Aim 2), with the dependent variable equal to 0 when diagnoses agreed and 1 when diagnoses disagreed, using BL vs. not BL. Model estimates are provided as prevalence ratios (PR) with 95% confidence intervals (CI). We limited the number of baseline covariates analyzed to about 5 events (discordance in this case) per covariate. We first ran univariate and multivariate models using the purposeful variable selection method². To do this we compared the frequency of baseline variables in Tables 1 and 2 between the overall cohort and the discordant group, as well as the concordant and discordant group. If the appeared to be a greater than

expected difference between either group, we identified this as a variable of interest and performed univariate and multivariate analysis.

For Aim 3 we defined survival time as the number of days from initial presentation at the UCI to the date of death, date of last visit, or 12 months, whichever came first. Note that date of tumor biopsy was not available for sufficient amount of patients, and therefore could not be used as start date for survival time. We used the Kaplan-Meier method to establish the survival of the entire cohort and to compare the survival of those with discordant diagnoses to those with concordant diagnoses. We used the log-rank test to compare survival in the discordant and concordant group.

5. Results

For calculations and stata commands please see **Appendix 2**.

5.1. Baseline Characteristics

Between July 15th 2012 and July 15th 2014 a total of 173 patients with suspected Burkitt Lymphoma (BL) were enrolled in the BL project. Of these 121 (70%) had histologically confirmed BL and 57 (33%) had pathology results from lab A and lab B and were eligible for our study.

Baseline cohort demographics and clinical characteristics for the eligible cohort are represented in Table 1 and Table 2 respectively. The median age of our eligible cohort was 7 years (range 1-19) with a male predominance (61%). The majority of patients had early stage disease (67% Ziegler stage A or B) and no clinical signs of CNS involvement on presentation (85%).

Table 1. Patient demographics

Baseline Demographics	Overall (n=57)	Concordant (n=39)	Discordant (n=18)
Age, median (range), n=50	7 (1-19)	7 (1-19)	9.75 (4-17)
Sex, n (%)			
Male	35 (61)	26 (67)	9 (50)
Female	22 (39)	13 (33)	9 (50)
Tribe, n(%)			
Musoga	16 (32)	9 (29)	7 (38.9)
Muganda	11 (22)	8 (25.8)	2 (11.1)
Ateso	6 (12)	4 (12.9)	2 (11.1)
Bagisu/Bagwere	4 (8)	2 (6.5)	2 (11.1)
Other ¹	13 (26)	8 (25.8)	5 (27.8)
Geographic region of residence, n (%)			
Northern Uganda	1 (1.9)	0	1 (5.56)
Eastern Uganda	28 (53.8)	17 (50)	11 (61.11)
Western Uganda	4 (7.6)	4 (11.7)	0
Central Uganda	19 (36.5)	12 (35.3)	6 (33.3)
Siblings, n (%)			
0	0	0	0
1-2	6 (12.5)	3 (9.38)	3 (18.75)
3-4	14 (29.2)	9 (28.12)	5 (31.25)
≥5	28 (58.3)	19 (62.4)	8 (50)

Primary caregiver, n (%)			
Mother	7 (13.2)	5 (14.3)	2 (11.1)
Father	4 (7.5)	1 (2.9)	3 (16.7)
Mother and Father	31 (58.5)	21 (60)	10 (55.6)
Other²	11 (20.8)	8 (22.8)	3 (16.7)
Distance from nearest major town to UCI in km, median (range), n=53	150 (22-534)	152 (22-534)	146 (22-313)
¹ Other tribes: Munyoro, Acholi/Langi, Munyankole, Munyarwanda, Mutooro, Samya/Japadhola, Munkonjo, Muruli, Kyojaimanyi			
² Other Primary care giver (n overall): grandmother (1); guardian (2); relative (1); mother, sister (1); mother, step-parent (1); mother, relative (1) father, mother, brother (1); father, mother, guardian (2); father, mother, grandmother (1)			

Table 2. Patient baseline characteristics at time of presentation to UCI

Baseline Characteristic	Eligible Cohort (n=57)	Concordant (n=39)	Discordant (n=18)
Stage at diagnosis, n (%)			
Early (A, B)	37 (67)	28 (74)	9 (53)
Late (C, D, AR)	18 (33)	10 (26)	8 (47)
Tumor Site, n (%)			
Jaw/face only	45 (80)	32 (84)	13 (72)

Abdomen only	3 (5.4)	1 (2.6)	2 (11)
Other site only	1 (1.8)	1 (2.6)	0
Jaw/face and abdomen	2 (3.6)	1 (2.6)	1 (5.6)
Jaw/face and other	4 (7.1)	3 (7.9)	1 (5.6)
Abdomen and other	1 (1.8)	0	1 (5.6)
Duration of symptoms, n (%)			
1-4 weeks	13 (41.9)	11 (45.8)	2 (28.6)
1-3 months	12 (38.7)	8 (33.3)	4 (57.1)
4-6 months	2 (6.4)	2 (8.3)	0
>6 months	4 (12.9)	3 (12.5)	1 (14.3)
ECOG status, n (%)			
0-2	46 (88)	33 (94)	13 (76)
3	6 (12)	2 (6)	4 (24)
CNS involvement, n (%)			
No	47 (85)	34 (92)	13 (72)
Yes	8 (15)	3 (8)	5 (28)
LDH, n (%)			
≤ 2 x upper limit of normal	18 (47)	16 (57)	2 (20)
> 2 x upper limit of normal	20 (53)	12 (43)	8 (80)
Platelet count, median (range), n=56	317 (36.7-1000)	315 (103-769)	318.5 (36.7-1,000)
Hemoglobin, median	10.35 (4.61-	10.35 (4.61-18.3)	10.25 (7.2-15.2)

(range), n=56

18.3)

5.2. Discordance

Of the eligible cohort a total of 18 (32%) patients had discordant diagnoses (Table 3) when comparing BL vs. “other” diagnoses. The most common differential diagnoses were rhabdomyosarcoma (RMS), lymphoblastic lymphoma (LL) and PNET (Table 3 and Table 4).

Table 3. Cross-tabulation of diagnoses (BL vs. other) from each of two laboratories

	Diagnosis from lab A, n=57		
Diagnosis from lab B, n=57	Burkitt Lymphoma	Other	Total
Burkitt Lymphoma	27	10	37
Other	8	12	20
Total	35	22	57

When further breaking down “other” diagnoses into ten diagnosis categories the percent of discordant cases increased to 44% (Table 5) as many of cases the “other” diagnoses were not the same between the two laboratories. The calculated kappa statistic for BL vs. “all combined” was 0.32 (95% CI 0.07-0.58) and for BL vs. the ten other diagnoses (see Table 5) the kappa statistic was 0.25 (95% CI 0.23-0.35).

Table 4. Diagnosis for lab 1 and lab 2 for patients with discordant diagnoses

Discordant group	Diagnosis Lab 1	Diagnosis Lab 2
1	BL	Benign Squamous Mucosa
2	BL	Benign tissue
3	BL	No malignancy
4	BL	PNET
5	BL	Squamous cell malignancy
6	BL	LL
7	BL	LL
8	BL	LL
9	Carcinoma	BL
10	HL	BL
11	PNET	BL
12	Poor tissue morphology	BL
13	RMS	BL
14	RMS	BL
15	RMS	BL
16	RMS	BL
17	Squamous malignancy	BL
18	LL	BL

Table 5. Cross-tabulation of diagnoses (BL vs. all other specified) from each of two laboratories

	Diagnosis from lab 1, n= 57										
Diagnosis from lab 2, n= 57	AL	BL	HL	KS	LL	O ¹	OB ²	OM ³	PNET	RB	RMS
AL	0	0	0	0	0	0	0	0	0	0	1
BL	0	27	1	0	1	1	1	1	1	0	4
HL	0	0	0	0	0	0	0	0	0	0	0
KS	0	0	0	0	0	0	0	0	0	0	0
LL	0	3	0	0	0	0	0	0	0	0	2
O ¹	0	0	0	0	0	0	0	0	0	0	0
OB ²	0	3	0	1	1	0	4	0	0	0	0
OM ³	0	1	0	0	0	0	0	1	0	0	0
PNET	0	1	0	0	0	0	0	0	0	0	0
RB	0	0	0	0	0	0	0	0	1	0	0
RMS	0	0	0	0	0	0	0	0	0	0	1

Table 5. Explanation of abbreviations: AL= Acute lymphoblastic leukemia, BL= Burkitt Lymphoma, HL= Hodgkin Lymphoma, KS=Kaposi sarcoma, LL= Lymphoblastic lymphoma, O=Other, OB=Other benign, OM= Other malignant, PNET= peripheral neuroectodermal tumor, RB= Retinoblastoma, RMS= Rhabdomyosarcoma

¹ Other: poor tissue morphology

²Other benign: Ameloblastoma, Benign squamous mucosa, Fibroma, Fibrous dysplasia, Giant cell granuloma, Left buccal sulcus exasion, No malignancy, Not malignant, Superficial squamous epithelium

³ Other malignant: Carcinoma, Immature teratoma, Squamous cell malignancy, Undifferentiated malignant neoplasm

The McNemar’s test for for BL vs. “other” showed a chi2 of 0.22 and a p-value of 0.64. Symmetry testing (Bowker’s test) to compare BL vs. the other none diagnosis categories demonstrated a chi2 of 23 (p-value 0.29) and testing for marginal homogeneity (Stuart-Maxwell) a chi 2 of 22.75 (p-value 0.54).

5.3. Characteristics associated with discordance

With the exception of age no notable demographic differences were observed between the discordant and concordant cohort (Table 1). Patients in the discordant group tended to be older compared to the concordant group (median age 7 vs. 9.75). Likewise patients with discordant diagnosis tended to have more advanced disease (early vs. late disease 74% vs. 26% for concordant and 53% vs. 47% for discordant), have CNS involvement (CNS negative vs. positive 92% vs. 8% for concordant and 72% vs. 28% for discordant) and have an elevated LDH at diagnosis (normal vs. elevated LDH 57% vs. 43% for concordant and 20% vs. 80% for discordant) (see Table 2). Due to these observed differences we restricted our analysis on association with discordance to: age, gender, stage, CNS involvement and LDH (see Table 5). Poisson regression analysis demonstrated a significant association between discordance and age (PR 1.09 per year older, 95% CI 1.021-1.18; $p=0.03$) as well as CNS involvement (PR 2.27, 95% CI 1.11-4.62; $p=0.025$) in unadjusted univariate models. There was a trend towards elevated LDH levels in the discordant group, however this did not meet statistical significance (PR 3.6, 95% CI 0.86-15.07; $p=0.079$). In multivariate models the PRs were attenuated and no longer statistically significant (Table 6).

Table 6. Poisson regression estimates of associations of baseline factors with diagnosis discordance.

Baseline covariate	Unadjusted PR (95% CI)	p-value	Adjusted PR ¹ (95% CI)	p-value
Age				
Per year older	1.09 (1.01-1.18)	0.03		
Male	0.63 (0.29-1.35)	0.23	0.55 (0.24-1.32)	0.183
Stage				
Early	1.0			
Late	1.83 (0.84-3.97)	0.13	1.92 (0.72-5.11)	0.19
CNS involvement				
No	1.0			
Yes	2.27 (1.11-4.62)	0.025	1.7 (0.58-4.98)	0.34
LDH				
≤ 2 x upper limit of normal	1.0			
> 2 x upper limit of normal	3.6 (0.86-15.07)	0.079	2.89 (0.65-12.79)	0.16

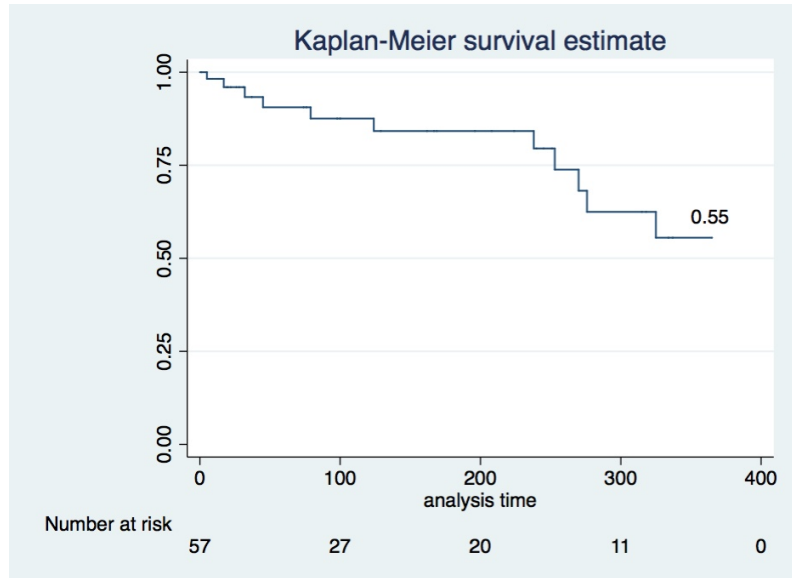
¹ Adjusted for Age

5.4. Impact of discordance on survival

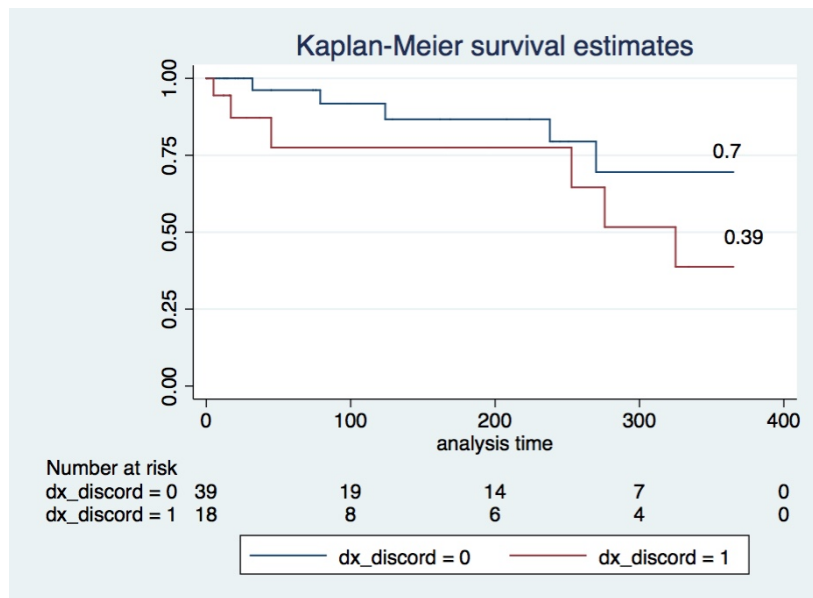
During the observation period there were 15 deaths events in the entire cohort, 7 in the concordant group and 8 in the discordant group. The risk ratio of death in the discordant group to the concordant group is therefore 3.63. One-year survival in the

entire cohort was 55%. However, survival at one year was 70% and 39% for concordant and discordant groups respectively (Graphs 1 and 2). Log rank test for equality of survivor function showed a non-statistically significant chi2 of 2.1 (p-value 0.15).

Graph 1: Survival all patients with two pathologic diagnoses



Graph 2: Survival patients with and without discordant diagnosis



6. Discussion

6.1. Baseline Characteristics of Cohort

Cohort characteristics, including age, gender, stage of disease and tumor location, of our cohort are comparable to what has been reported in the literature for endemic Burkitt Lymphoma in children, providing evidence that this is a representative cohort of patients with Burkitt Lymphoma. [22, 23] The majority of our patients are from Eastern Uganda, which is expected based on the catchment area of the Uganda Cancer Institute.

The percentage of patients with a discordant diagnosis for BL vs. other at 32% was considerably lower compared to previous reports by Orem et al.[21] This study reported a percentage of agreement of 36% for BL vs. other diagnoses, which would correlate to a percentage of disagreement of 64%, approximately double of what we are seeing in our study. Even when looking at BL vs. ten other diagnoses our percentage of disagreement only increases to 44%. Our calculated kappa statistics is correspondingly higher (0.32 compared to 0.11), though overall still representing a fair interobserver agreement (Cohen's interpretation of kappa statistics, see Appendix 2, Figure1). The differences we are seeing between the two studies may partly be due to the time of the study, as the study by Orem was between 2004 and 2008 and our study covers years 2012-2014. During this time pathology services may have improved. Our study, unlike the study by Orem et al., also did not work with a reference lab in a country with higher resources. Both of our pathology labs were in Uganda, though the pathologist from one of the labs was practicing in the

United States. Finally, in the study by Orem et al. the reference lab in the Netherlands had access to IHC staining, which neither of the labs involved in our study had. All of these factors may have contributed to the higher discordance seen in the study by Orem et al. Another study from St. Mary Hospital, Lacor, Uganda, documented a percent agreement of 82% (range 58-88% due to non-evaluable tissue biopsies), resulting in a percent disagreement of 18%. [20]The fact that this study had many non-evaluable samples (n=19) may in part explain the discrepancy in the numbers we are seeing, as in our study no samples were deemed non-evaluable.

What is interesting is that the main differential diagnoses in both the aforementioned studies were other Non-Hodgkin diseases (NHL) and Hodgkin disease. Other cancers were rare. Though lymphoblastic lymphoma (a NHL) was seen in our study, solid tumors like rhabdomyosarcoma (RMS) and PNET were more common differential diagnoses in our cohort. Likewise there was a high rate of non-malignant tumors seen in our cohort. This discrepancy may be due to the fact that the inclusion criteria for patients in the different cohorts were different. For example, for the study by Orem et al. the inclusion criteria for a clinical diagnosis of suspected BL required a facial tumor, whereas this was not a requirement for our study. RMS and PNET, seen more commonly in our study, may present as abdominal tumors as well.

6.2. Factors associated with discordant diagnoses

To date there are no publications to our knowledge looking at associations of a discordant diagnosis with baseline patient characteristics. We postulate that a discordant diagnosis increases the likelihood of a misdiagnosis, however does not necessarily equate to a misdiagnosis. When trying to understand associations between baseline factors and a discordant diagnosis we must consider two main aspects: 1. how a baseline factor may influence the pathologist in terms of the differential diagnoses they consider; and 2. the epidemiology of diseases and what the likelihood of a certain diagnosis is. These factors may work in opposite directions at times, making it more difficult to understand the associations.

We found statistically significant associations between the likelihood of having a discordant diagnosis and being of older age and having clinical symptoms of CNS involvement. These associations were however small. The median age was 7 years for the entire cohort, and was about the same for patients without discordant diagnosis. For patients with discordant diagnosis the median age was older, around 9 years, and the risk of a discordant diagnosis increased statistically significantly for per year older. In the literature the median age of patients with endemic BL has been reported as around 7 years. The pathologists were not blinded to the age of the patients, and therefore the knowledge of an older age, has the potential to influence the diagnoses a pathologist considers. On the other hand from an epidemiologic standpoint the likelihood of an older patient having BL is also lower and other differential diagnoses become more likely. Therefore the association of age and discordant diagnosis is comprehensible and should be considered when a patients with suspected BL present with suspected BL.

The association of CNS disease involvement is less clear. CNS involvement for this study was defined as any symptom of CNS involvement, but not by laboratory findings of CNS involvement. This means that a patient would have had symptoms like seizures, cranial nerve, sensory or motor deficits, for example. CNS involvement is certainly seen in advanced stages of endemic BL, however the expected frequency of CNS involvement is not well described for endemic BL. For sporadic BL a CNS involvement of 10-20% would be expected, which is what we are seeing in the whole cohort and within the concordant group. In the discordant group the CNS involvement is higher than expected. This could be explained by the fact that other diseases, like lymphoblastic lymphoma or RMS, are more likely for this group, creating an opportunity for a misdiagnosis by the pathologist. On the other hand if CNS involvement is associated with later disease stages, this may mean that the tumor tissue obtained from biopsy has more necrotic areas, making it harder to analyze for the pathologist. The information of clinical CNS involvement may not be readily available to the pathologist and therefore may or may not influence the differential diagnoses considered. The effect was no longer statistically significant when adjusted for age. Therefore age may play into the likelihood of having CNS involvement.

Increased LDH had a non-statistically significant association with discordance. In general elevated LDH levels would be seen in more advanced stages of BL and predict a worse disease outcome. However elevated LDH levels are not specific to any one disease, and can also be seen in other NHLs, other cancers and non-malignant diseases. If an LDH level was increased in a patient and the pathologist

was aware of this baseline clinical laboratory this may bias his or her differential diagnosis towards the consideration of BL. Knowing that elevated LDH is not specific or sensitive to BL could then explain why patients with elevated LDH would be more likely to have a discordant diagnosis.

6.3. Impact of discordance on survival

The one-year overall survival of 55% for this cohort of pediatric patients with suspected eBL was comparable to what has been previously reported in the literature for countries in Sub-Saharan Africa [9, 24-26], and remains low compared to survival for children with sporadic BL treated in higher income countries.[5, 27] Similar to associations with discordant diagnosis, to our knowledge there is no literature on how a discordant diagnosis impacts survival of patients with endemic BL. The relative risk of death in the group with discordance was higher compared to patients with a concordant diagnosis and the overall survival was lower. However, this did not meet statistical significance. With the small sample size, it is likely that this study was underpowered to be able to show a statistically significant difference in survival, and a larger sample size may actually show a survival advantage for patients with a concordant diagnosis. Intuitively it makes sense to assume that a patient with a less definitive diagnosis, would not fair as well on a treatment protocol (COM in the case of endemic BL) that may not be as effective for the treatment of their disease. We also have to consider that these results are confounded by baseline factors associated with discordance, like older age, elevated

LDH and CNS involvement, all of which are associated with worse outcomes on patients with NHLs, like BL. In larger studies we could adjust for these confounders.

6.4. Implications of findings

Our study is limited by the small sample size, which causes our study to be underpowered in its ability to show associations and/or effects. The sample size also limits the number of associations we can test for, and therefore some of these may have been omitted. There is also a risk of confounding by baseline factors that are associated with discordance and another baseline variable (for example age is associated with worse outcomes in children with BL and age was associated with discordance). In our multivariate model the associations between discordance and baseline factors were all attenuated when adjusted for age.

Another limitation of our study is that it includes a survival analysis of patients who live in a low-resource setting, where the loss to follow up is often high. While we have been following our patients with the help of case managers and our loss to follow up is comparably low at 6%, this could influence the results of our study.

While our study has been following patients systematically some data were missing, and limited our analysis. Specifically, for our survival analysis we used the date of presentation to the UCI as the start date. However, some patients already had a biopsy performed prior to presenting, and some patients did not have a biopsy performed until they presented to the UCI. Patients needed to have a biopsy result to be eligible for this study and therefore patients who presented without a diagnosis to the UCI could have had an immortal time bias, as they had to survive

until they underwent a biopsy, while patients presenting already with a biopsy would not be subject to this bias. As pathology results may routinely take a week to several weeks to be reported, it is possible that this effect could influence the analysis.

Despite these limitations we were able to show that discordance occurs at a high rate in the setting of low-resources. Diagnostic uncertainty affects many levels of the healthcare system. Most importantly a patient who receives two diagnoses from different pathology laboratories may experience a significantly higher level of stress going through the diagnosis and treatment of their disease. They may also lose trust in the medical system and their caregivers. Treating physicians are forced to rely more on their clinical judgment, which may be difficult for more junior physicians. Having a high level of discordance in their patient population may also lead providers not to trust their pathologist, and may lead them to doubt other diagnoses made. For pathologists themselves knowing that their diagnosis contrasts with the diagnosis of another pathologist may also lead to frustration with their healthcare system and the resources and training provided to them and their colleagues. Therefore these results should make a strong case for improving resources for pathologist in resource-limited settings. A cost effectiveness analysis of this would be helpful in determining the financial impact of a discordant diagnosis on the healthcare system.

Results for Aim 2 and Aim3 looking at baseline factors associated with discordant diagnosis and impact on overall survival, should best be interpreted as preliminary

findings as the small numbers in this study limits the interpretation. Therefore they should be confirmed in a larger study. The associations of age and elevated LDH with discordance that we demonstrated in our analysis can be used when deciding which patients may need more diagnostic scrutiny than others. For example patients, who are significantly older than the median age of 7 years, may be selected to undergo further diagnostic testing with immunohistochemistry (IHC) on their tissue samples. This would increase the diagnostic accuracy for patients at risk for discordance and at the same time not overburden the medical system by performing IHC on every patient.

In order to be able to make more definitive statements regarding associations with discordance a larger sample size is needed. As more data become available to us we will continue our analysis. However, discordance is merely a maker for possible misdiagnosis and concordance does not guarantee the right diagnosis in a system that has a high rate of discordance. Therefore a more definitive study would be to perform IHC on the tumor tissue samples and have a pathologist review the slides to make a tie-breaker diagnosis. This way we would have information on the true rate of misdiagnosis. We could then perform similar analysis looking at associations on baseline factors with misdiagnosis and the impact of misdiagnosis on overall survival. This study is currently in the planning stages at our institution.

While this study and the proposed follow-up study aim at identifying existing problems, and may help in providing some guidance in terms of which patients need more extensive diagnostic testing, including IHC staining, this may not offer feasible

long-term solutions for healthcare systems that are overburdened with costs. While identifying the problem is a first important step, we must also work on providing solutions that are feasible in this setting. Cheaper and more reliable tests should be made available to countries with limited resources that would help increase diagnostic accuracy. For example, for endemic BL, we could take advantage of the fact that close to 100% of tumors are associated with EBV, by including a staining for EBV in a limited IHC panel. As many tumors in low-resource settings are associated with viruses and other infectious agents this approach could also apply to other tumors, like Kaposi sarcoma (HHV8) and nasopharyngeal carcinoma (EBV). Testing for HPV is already being used in the diagnosis of cervical cancer.

7. Appendixes

Appendix 1. Patient intake forms (link to files)

[../BL/BL project/Patient Forms/Current forms/BL CRFs--2013-08-04.pdf](https://bl/blproject/PatientForms/Currentforms/BLCRFs--2013-08-04.pdf)

Appendix 2. Calculations and stata commands

Calculations and stata commands

Background: The summaries shown in this report are preliminary analyses intended for the write-up of my thesis “Frequency, Predictors and Sequelae of Discordant Histopathologic Diagnoses Among Patients with endemic Burkitt Lymphoma presenting for care in a limited resource setting”. The results are based on secondary data analysis of the BL project database. Data was exported from the salesforce database by Innocent Mutyaba, deidentified and submitted to me for analysis. Excel data was imported into stata for further analysis. Support in the analysis was provided by Elizabeth Krantz, statistician.

Eligibility: We included patients enrolled into the BL project between July 2012 and July 2014, who had at least two pathologic diagnoses from different pathology laboratories and were suspected to have BL based on physical exam findings. 174 patients with suspected BL were enrolled in the project during this time, of which 121 had confirmed BL (at least one diagnosis of BL by histopathology and the diagnosis of BL by physical exam findings). A total of 57 patients met our inclusion criteria. Baseline cohort characteristics are missing for some patients, therefore numbers do not always add up to 57 (see Table 0.1).

Definitions:

1. Suspected eBL: patient enrolled in the BL project and worked up for possible eBL.

2. Discordant diagnosis: Discordance will be defined as any participating children with a diagnosis from one local pathology lab that differed from the diagnosis given at the other local pathology lab.
3. Early Stage: Ziegler stages A or B
4. Late Stage: Ziegler stages C, D or AR

Results:

1. Baseline Characteristics

First, we will summarize baseline characteristics, overall and by discordance. For continuous variables that are missing for some patients, we will give the number of observations with data in column 1. For categorical variables, all categories are shown so that the extent of missing data can be deduced from those totals.

Table 0.1. Patient demographics

Baseline Demographics	Overall (n=57)	Concordant (n=39)	Discordant (n=18)
Age, median(range), n=50	7 (1-19)	7 (1-19)	9.75 (4-17)
Sex, n (%)			
Male	35 (61)	26 (67)	9 (50)
Female	22 (39)	13 (33)	9 (50)
Tribe, n(%)			
Musoga	16 (32)	9 (29)	7 (38.9)
Muganda	11 (22)	8 (25.8)	2 (11.1)
Ateso	6 (12)	4 (12.9)	2 (11.1)
Bagisu/Bagwere	4 (8)	2 (6.5)	2 (11.1)
Other¹	13 (26)	8 (25.8)	5 (27.8)
Geographic region of residence, n (%)			
Northern Uganda	1 (1.9)	0	1 (5.56)
Eastern Uganda	28 (53.8)	17 (50)	11 (61.11)
Western Uganda	4 (7.6)	4 (11.7)	0

Central Uganda	19 (36.5)	12 (35.3)	6 (33.3)
Siblings, n (%)			
0	0	0	0
1-2	6 (12.5)	3 (9.38)	3 (18.75)
3-4	14 (29.2)	9 (28.12)	5 (31.25)
≥5	28 (58.3)	19 (62.4)	8 (50)
Primary caregiver, n (%)			
Mother	7 (13.2)	5 (14.3)	2 (11.1)
Father	4 (7.5)	1 (2.9)	3 (16.7)
Mother and Father	31 (58.5)	21 (60)	10 (55.6)
Other ²	11 (20.8)	8 (22.8)	3 (16.7)
Distance from nearest major town to UCI in km, median (range), n=53	150 (22-534)	152 (22-534)	146 (22-313)
¹ Other tribes: Munyoro, Acholi/Langi, Munyankole, Munyarwanda, Mutooro, Samya/Japadhola, Munkonjo, Muruli, Kyojaimanyi			
² Other Primary care giver (n overall): grandmother (1); guardian (2); relative (1); mother, sister (1); mother, step-parent (1); mother, relative (1) father, mother, brother (1); father, mother, guardian (2); father, mother, grandmother (1)			

Table 0.2. Patient baseline characteristics at time of presentation to UCI

Baseline Characteristic	Eligible Cohort (n=57)	Concordant (n=39)	Discordant (n=18)
Stage at diagnosis, n (%)			
Early (A, B)	37 (67)	28 (74)	9 (53)
Late (C, D, AR)	18 (33)	10 (26)	8 (47)
Tumor Site, n (%)			
Jaw/face only	45 (80)	32 (84)	13 (72)
Abdomen only	3 (5.4)	1 (2.6)	2 (11)

Other site only	1 (1.8)	1 (2.6)	0
Jaw/face and abdomen	2 (3.6)	1 (2.6)	1 (5.6)
Jaw/face and other	4 (7.1)	3 (7.9)	1 (5.6)
Abdomen and other	1 (1.8)	0	1 (5.6)
Duration of symptoms, n (%)			
1-4 weeks	13 (41.9)	11 (45.8)	2 (28.6)
1-3 months	12 (38.7)	8 (33.3)	4 (57.1)
4-6 months	2 (6.4)	2 (8.3)	0
>6 months	4 (12.9)	3 (12.5)	1 (14.3)
ECOG status, n (%)			
0-2	46 (88)	33 (94)	13 (76)
3	6 (12)	2 (6)	4 (24)
CNS involvement, n (%)			
No	47 (85)	34 (92)	13 (72)
Yes	8 (15)	3 (8)	5 (28)
LDH, n (%)			
≤ 2 x upper limit of normal	18 (47)	16 (57)	2 (20)
> 2 x upper limit of normal	20 (53)	12 (43)	8 (80)
Platelet count, median (range), n=56	317 (36.7-1000)	315 (103-769)	318.5 (36.7-1,000)
Hemoglobin, median (range), n=56	10.35 (4.61-18.3)	10.35 (4.61-18.3)	10.25 (7.2-15.2)

Explanation of modifications to proposal outline for table 0.1 and 0.2:

Of note, for table 0.1 I excluded “nationality” as all patients were Ugandan. The tribes listed are modified based on the frequency seen in our population. “Other” was taken out of “Geographic region” as no participants were from other regions.

“Primary caregiver” was modified based on the caregiver structure see among our patients.

For table 0.2, “tumor size at presentation”, “B-Symptoms present”, “Tumor lysis syndrome present” and “HIV status” were not available for this analysis, as there were too many observations missing. The following variables were added: “Stage at diagnosis”, “ECOG status”, “CNS involvement”, “Platelet count” and “Hemoglobin”. “Duration of symptoms” presentation was modified from median and range to specific time periods as provided by the dataset.

Results:

Based on the differences seen in proportion of baseline variables in tables 0.1 and 0.2 between discordant and concordant groups compared to the entire group we decided to test the following variables for their association with discordance: age, sex, stage at diagnosis, CNS involvement and LDH level (see table 2.1).

Aim 1. To determine the discordance of diagnosis among children presenting with suspected eBL in Uganda

We will then tabulate patients by their discordance status (table 1.0):

Table 1.0 Discordance status of patients

Discordance BL vs. other diagnosis between lab 1 and 2	Frequency	Percent	Cum.
0	39	68.42	68.42
1	18	31.58	100.00
Total	57	100.00	100.00

Results Table 1.0:

There are a total of 57 patients with two diagnoses from the two pathology laboratories. A total of 18 (32%) patients had discordant diagnoses.

Next we cross-tabulate the frequency of diagnoses by each of the 2 laboratories (Table 1.1- 1.3), first BL vs. all other (Table 1.1), then BL vs. the most common diagnoses and “other” (table 1.2), then with more specifics regarding some of the “other” diagnoses (Table 1.3).

Table 1.1 Cross-tabulation of diagnoses from each of two laboratories (BL vs. all other)

	Diagnosis from lab 1, n=57		
Diagnosis from lab 2, n= 57	Burkitt Lymphoma	Other ¹	Total
Burkitt Lymphoma	27	10	37
Other ¹	8	12	20
Total	35	22	57

¹“Other” include: Acute lymphoblastic leukemia, Ameloblastoma, Benign squamous mucosa, Carcinoma, Fibroma, Fibrous dysplasia, Giant cell granuloma, Hodgkin Lymphoma, Immature teratoma, Kaposi sarcoma, Left buccal sulcus exasion, Lymphoblastic lymphoma, No malignancy, Not malignant, Peripheral neuroectodermal tumor, Poor tissue morphology, Retinoblastoma, Rhabdomyosarcoma, Superficial squamous epithelium, Squamous malignancy, Undifferentiated malignant neoplasm

Table 1.2 Cross-tabulation of diagnoses from each of two laboratories (BL vs. RMS, LL and Other)

	Diagnosis from lab 1, n= 57			
Diagnosis from lab 2, n= 57	Burkitt Lymphoma	Other ¹	Rhabdomyosarcoma	Lymphoblastic Lymphoma
Burkitt Lymphoma	27	5	4	1
Other ¹	5	7	0	1
Rhabdomyosarcoma	0	0	1	0
Lymphoblastic Lymphoma	3	0	2	0

¹“Other” include: Acute lymphoblastic leukemia, Ameloblastoma, Benign squamous mucosa, Carcinoma, Fibroma, Fibrous dysplasia, Giant cell granuloma, Hodgkin Lymphoma, Immature teratoma, Kaposi sarcoma, Left buccal sulcus exasion, No malignancy, Not malignant, Peripheral neuroectodermal tumor, Poor tissue morphology, Retinoblastoma, Superficial squamous epithelium, Squamous malignancy, Undifferentiated malignant neoplasm

Table 1.3 Cross-tabulation of diagnoses from each of two laboratories (BL vs. all other specified)

	Diagnosis from lab 1, n= 57										
Diagnosis from lab 2, n= 57	AL	BL	HL	KS	LL	O ¹	OB ²	OM ³	PNET	RB	RMS
AL	0	0	0	0	0	0	0	0	0	0	1
BL	0	27	1	0	1	1	1	1	1	0	4
HL	0	0	0	0	0	0	0	0	0	0	0
KS	0	0	0	0	0	0	0	0	0	0	0
LL	0	3	0	0	0	0	0	0	0	0	2
O ¹	0	0	0	0	0	0	0	0	0	0	0
OB ²	0	3	0	1	1	0	4	0	0	0	0
OM ³	0	1	0	0	0	0	0	1	0	0	0
PNET	0	1	0	0	0	0	0	0	0	0	0
RB	0	0	0	0	0	0	0	0	1	0	0
RMS	0	0	0	0	0	0	0	0	0	0	1

Table 1.3 Explanation of abbreviations: AL= Acute lymphoblastic leukemia, BL= Burkitt Lymphoma, HL= Hodgkin Lymphoma, KS=Kaposi sarcoma, LL= Lymphoblastic lymphoma, O=Other, OB=Other benign, OM= Other malignant, PNET= peripheral neuroectodermal tumor, RB= Retinoblastoma, RMS= Rhabdomyosarcoma

¹ Other: poor tissue morphology

²Other benign: Ameloblastoma, Benign squamous mucosa, Fibroma, Fibrous dysplasia, Giant cell granuloma, Left buccal sulcus exasion, No malignancy, Not malignant, Superficial squamous epithelium

³ Other malignant: Carcinoma, Immature teratoma, Squamous malignancy, Undifferentiated malignant neoplasm

Results Tables 1.1-1.3:

Table 1.1 demonstrates that in 27 cases both pathology labs agreed on the diagnosis of BL. In 12 cases they agreed that it was not BL (“other”), however “other” may not be the same diagnosis for both labs. Therefore the number of concordance may be overestimated in this case (39). This becomes clearer in tables 1.2 and 1.3, where there is more detail given for the “other” diagnoses. For table 1.2 the number of concordant cases decreases to 35, and in table 1.3 to 33.

Explanation for modification from proposal outline:

Table 1.0 was added to demonstrate the number of discordant and concordant cases and how we establish the frequency discordance. Table 1.1 was modified into three different versions (Tables 1.1-1.3) to establish the best way to present the data visually but also to highlight the degree of discordance.

Inferential Analysis: We will compute the kappa statistic, with 95% confidence intervals (CI) to measure agreement between the 2 pathology labs, using BL vs. “other” (Table 1.1). Then we will test the kappa for BL vs. the 10 diagnoses outlined in Table 1.3. To test whether the 2 diagnoses are significantly different, we will use tests appropriate for paired data, in this case we will compare a 2-category diagnosis of eBL versus not-eBL using McNemar’s test.

1. Kappa for BL vs. “other” (table 1.1)

Stata command for kapci: kapci dx_bl_surg dx_bl_mu

Kappa (95% CI) = 0.322 (0.070 - 0.575)

2. Kappa for BL vs. 10 other diagnoses (table 1.3)

Stata command: kapci dx_any_surg dx_any_mu

Kappa (95% CI) = 0.245 (0.226-0.345)

Based on Cohen’s kappa this indicated **fair agreement** for both scenarios. As expected the kappa for scenario 2 is smaller, as the number of observed agreements decreased.

Figure 1. Interpretation of Kappa statistic

Table 2

Interpretation of Kappa

	Poor	Slight	Fair	Moderate	Substantial	Almost perfect
Kappa	0.0	.20	.40	.60	.80	1.0

<u>Kappa</u>	<u>Agreement</u>
< 0	Less than chance agreement
0.01–0.20	Slight agreement
0.21– 0.40	Fair agreement
0.41–0.60	Moderate agreement
0.61–0.80	Substantial agreement
0.81–0.99	Almost perfect agreement

Taken from: Viera AJ, Garrett, JM, Understanding Interobserver Agreement: The Kappa Statistic. *Fam Med.* 2005;37(5):360-3.

3. McNemar’s test for BL vs. “other” (Table 1.1)

Stata command for McNemar’s test: `mcc dx_bl_surg dx_bl_mu`

Table 1.5 McNemar’s test for BL vs. “other” diagnosis

	Cases		
	Exposed	Unexposed	Total
Controls			
Exposed	27	10	37
Unexposed	8	12	20
Total	35	22	57

McNemar's $\chi^2(1) = 0.22$ Prob > $\chi^2 = 0.6374$

Exact McNemar significance probability = 0.8145

Based on the McNemar’s $\chi^2(1)$ of 0.22 and a p value of 0.64, the results of the two pathology labs are not significantly different. This is probably expected, as it would be unusual if we would be able to find a statistically significant difference between the two laboratory diagnosis. In this case the kappa is

probably the better statistic to show, as it shows the agreement between the two labs.

Aim 2. To identify baseline factors associated with discordance of diagnosis among children presenting with suspected eBL in Uganda

Inferential Analyses: Based on preliminary data we expect that discordance to be a frequent event (>10% of all patients with two diagnoses). Therefore, to identify baseline factors associated with discordance, we will use Poisson regression with the dependent variable equal to 0 when diagnoses agree and 1 when diagnoses disagree, using BL vs. not BL. Model estimates will be provided as prevalence ratios (PR) with 95% confidence intervals (CI). Because we only have 18 discordant cases, we will limit the number of baseline covariates to those variables that appeared to show some differences descriptively, as well as important clinical variables. Based on table 2.1 we will restrict this to: **age, gender, stage, CNS involvement and LDH**. Because we have more variables than we can accommodate while ensuring a minimum of 5 events per independent variable, we will simply present unadjusted and age-adjusted model estimates. We will not adjust for stage, as stage may be colinear with elevated LDH and CNS involvement.

Table 2.1 Patient demographics and clinical characteristics by discordance of diagnoses between Lab A and Lab B

Baseline Characteristic	Concordant (n=39)	Discordant (n=18)
Age, median(range)	7 (1-19)	9.75 (4-17)
Sex, n (%)		
Male	26 (67)	9 (50)
Female	13 (33)	9 (50)
Stage at diagnosis, n (%)		
Early (A, B)	28 (74)	9 (53)
Late (C, D, AR)	10 (26)	8 (47)
Tumor Site, n (%)		

Jaw/face only	32 (84)	13 (72)
Abdomen only	1 (2.6)	2 (11)
Other site only	1 (2.6)	0
Jaw/face and abdomen	1 (2.6)	1 (5.6)
Jaw/face and other	3 (7.9)	1 (5.6)
Abdomen and other	0	1 (5.6)
Duration of symptoms, n (%)		
1-4 weeks	13 (41.9)	11 (45.8)
1-3 months	12 (38.7)	8 (33.3)
4-6 months	2 (6.4)	2 (8.3)
>6 months	4 (12.9)	3 (12.5)
ECOG status, n (%)		
0-2	33 (94)	13 (76)
3	2 (6)	4 (24)
CNS involvement, n (%)		
No	34 (92)	13 (72)
Yes	3 (8)	5 (28)
LDH, n (%)		
≤ 2 x upper limit of normal	16 (57)	2 (20)
> 2 x upper limit of normal	12 (43)	8 (80)
Platelet count, median (range), n=56	315 (103-769)	318.5 (36.7-1,000)
Hemoglobin, median (range), n=56	10.35 (4.61-18.3)	10.25 (7.2-15.2)

Stata commands:

1. Age: poisson dx_discord age, vce(robust) irr
2. Gender: poisson dx_discord male, vce(robust) irr

3. Late stage: poisson dx_discord stage_late, vce(robust) irr
4. CNS involvement: poisson dx_discord cns, vce(robust) irr
5. LDH elevated: poisson dx_discord ldh_hi, vce(robust) irr

Table 2.2. Poisson regression estimates of associations of baseline factors with diagnosis discordance.

Baseline covariate	Unadjusted PR (95% CI)	p-value	Adjusted PR ¹ (95% CI)	p-value
Age				
Per year older	1.09 (1.01-1.18)	0.03		
Male	0.63 (0.29-1.35)	0.23	0.55 (0.24-1.32)	0.183
Stage				
Early	1.0			
Late	1.83 (0.84-3.97)	0.13	1.92 (0.72-5.11)	0.19
CNS involvement				
No	1.0			
Yes	2.27 (1.11-4.62)	0.025	1.7 (0.58-4.98)	0.34
LDH				
≤ 2 x upper limit of normal	1.0			
> 2 x upper limit of normal	3.6 (0.86-15.07)	0.079	2.89 (0.65-12.79)	0.16

¹Adjusted for age

Results Table 2.2 Two of the PRs reach statistical significance: Age with a p value of 0.03 per year older and CNS involvement with a p value of 0.025. For age the PR is small, however this has to be seen in the context of an increase per year. The PR for CNS involvement is also small at 2.27. Elevated LDH has the highest PR however does not reach statistical significance.

Aim 3. To determine how discordance in pathology results impact the overall survival (OS) of patients with eBL in a setting with limited resources.

First we will tabulate death events using stata command for the overall cohort and then for the concordant and discordant groups separately (see tables 3.1-3.3):

1. Eligible cohort: tab vital_st
2. Concordant group: tab vital_st if dx_discord==0
3. Discordant group: tab vital_st if dx_discord==1

Table 3.1. Deaths events in entire cohort

Vital_st	Freq.	Percent	Cum.
Death	15	26.32	26.32
Alive	42	73.68	100.00
Total	57	100.00	100.00

Table 3.2. Deaths events for patients with concordant diagnosis

Vital_st	Freq.	Percent	Cum.
Death	7	17.95	17.95
Alive	32	73.68	100.00
Total	39	100.00	100.00

Table 3.3. Deaths events for patients with discordant diagnosis

Vital_st	Freq.	Percent	Cum.
Death	8	44.44	44.44
Alive	10	55.56	100.00
Total	18	100.00	100.00

Results Tables 3.1-3.3:

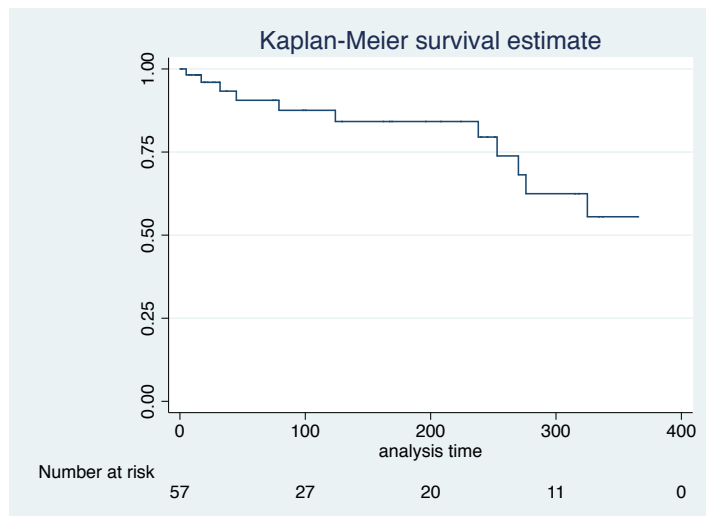
There were a total of 15 deaths in the entire cohort, with more than 50% (n=8) of deaths occurring in the discordant group. Note that these death are for the entire follow up period and we will censor at 12 months for Kaplan Meier curves and Log rank testing.

Inferential Analysis: For the analysis of survival we will first perform a Kaplan-Meier survival estimate for the entire cohort and then break it down based on discordance status. We will set the time variable in stata to survdays_1yr, and set the failure variable to died_1yr. Survival days is calculated from the time of presentation at the UCI until death or the time of censoring or 12 months.

Stata commands:

1. `stset survdays_1yr, failure(died_1yr) scale(1)`
2. `sts graph, risktable`

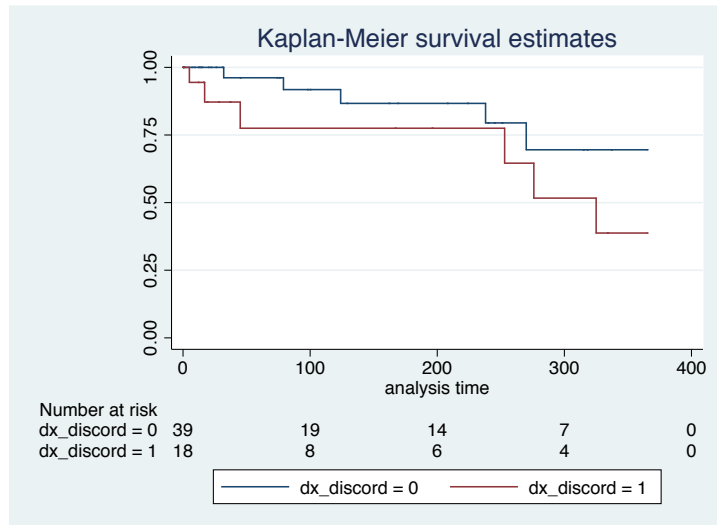
Graph 1: Survival all patients with two pathologic diagnoses



stata command for survival by discordance:

3. `sts graph, by(dx_discord) risktable`

Graph 2: Survival patients with and without discordant diagnosis



Results Kaplan Meier curves:

There seems to be a difference between survival in patients with and without discordance, though we would have to test if the difference in survival is significant.

To test if the survival time is significantly different we will use the log rank test for equality:

Stata command:

4. sts test dx_discord
5. failure _d: died_1yr
6. analysis time _t: survdays_1yr

Table 3.4 Log-rank test for equality of survivor functions

Dx_discord	Events observed	Events expected
0	5	7.26
1	6	3.74

Total	11	11.00
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chi2(1) = 2.10

Pr>chi2 = 0.1468

Though there are more deaths in the discordant group than expected the difference is not statistically significant with a p-value of 0.15. This may be due to small numbers.

Cox proportional hazards models to test for an association between discordant diagnosis and survival (Table 3.5).

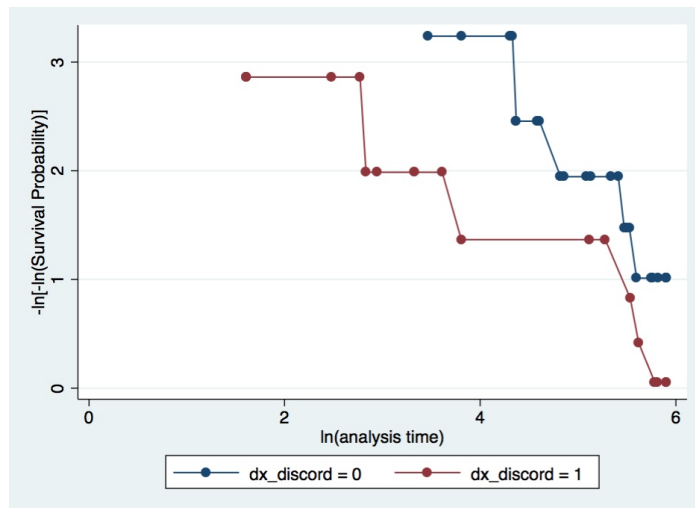
non-zero slope in the Schoenfeld residuals (“estat phtest” command in Stata).

Table 3.5 Cox regression estimates of associations between discordant diagnosis between pathology lab A and B and survival.

Baseline covariate	Unadjusted HR (95% CI)	p-value	Adjusted HR (95% CI) ¹	p-value
Discordant diagnosis	2.37 (0.71-7.85)	0.159	0.76 (0.07-7.91)	0.82

¹ Adjusted for age

Graph 3 Test of proportional hazards assumption



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