original reports abstract

Accuracy of Pathologic Diagnosis in Patients With Lymphoma and Survival: A Prospective Analysis From Botswana

Fallon E. Chipidza, MD[1,2]; Mukendi K. A. Kayembe, MD[3,4]; Isaac Nkele, BA[5]; Jason A. Efstathiou, MD, DPhil[2]; Bruce A. Chabner, MD[6]; Jeremy Abramson, MD[6]; Scott L. Dryden-Peterson, MD[7]; and Aliyah R. Sohani, MD[8]

PURPOSE With intense HIV epidemics, southern African countries have a high burden of classic Hodgkin lymphoma (CHL) and non-Hodgkin lymphoma (NHL). However, suboptimal access to pathology resources limits subtype classification. We sought to assess the diagnostic accuracy of specimens classified as lymphoma and to determine association between discordant pathologic diagnosis and overall survival.

METHODS Seventy patients with CHL or NHL and treated at three Botswana hospitals from 2010 to 2016 were analyzed. Local pathologic assessment relied primarily on morphology. All cases underwent secondary US hematopathology review, which is considered gold standard.

RESULTS The median follow-up was 58 months. The overall reclassification rate was 20 of 70 cases (29%). All 20 CHL cases were correctly classified in Botswana, and mixed cellularity was the most common subtype, diagnosed in 11 (55%) cases. Of 47 confirmed NHL cases, diffuse large B-cell lymphoma was the final US diagnosis in 28 cases (60%), another aggressive B-cell NHL in nine (19%), an indolent B-cell NHL in six (13%), and T-cell NHL in four (9%). Common types of diagnostic discordance included NHL subtype reclassification (11 of 20, 55%) and CHL reclassified as NHL (7 of 20, 35%). Concordant versus discordant diagnosis after secondary review was associated with improved 5-year overall survival (60.1% *v* 26.3%, $P = .0066$). Discordant diagnosis was independently associated with increased risk of death (adjusted hazard ratio 2.733; 95% CI, 1.102 to 6.775; $P = .0300$) even after stratifying results by CHL versus NHL.

CONCLUSION In this single prospective cohort, discordant pathologic diagnosis was associated with a nearly three-fold increased risk of death. Limited access to relatively basic diagnostic techniques impairs treatment decisions and leads to poor patient outcomes in low-resource countries.

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INTRODUCTION

With nearly one fifth of the adult population living with HIV, Botswana is among the top three countries with the highest HIV prevalence globally.[1] Adoption of a nationwide program to make antiretroviral therapy freely available to all patients with HIV has led to a dramatic decline in mortality, but a steady rise in HIV-associated malignancies as the population living with HIV ages.[2] The three most common HIV-associated cancers in this population are lymphoma, cervical cancer, and Kaposi sarcoma, together accounting for nearly half of all malignant cases in Botswana.[3]

The histologic profiles of lymphomas in various African countries have been reported in several studies.[4-6] These studies have demonstrated that aggressive lymphoma subtypes, including diffuse large B-cell lymphoma (DLBCL) and Burkitt lymphoma (BL), constitute most lymphomas in people living with HIV. Classic Hodgkin lymphoma (CHL), another HIV-related malignancy, has also seen a sharp increase in incidence with the introduction of antiretroviral therapy.[6] However, studies specifically examining pathologic subtypes of lymphoma in African countries that support wide-scale HIV treatment programs are limited.

The precise classification of histologic subtype of lymphomas has important implications for the choice of therapy and patient outcomes. The most effective regimens for various subtypes of lymphoma differ significantly in terms of drug combinations and use of B-cell–specific monoclonal antibodies. We retrospectively assessed the diagnostic accuracy of all new cases of lymphoma diagnosed at three tertiary hospitals in Botswana by performing secondary pathology review with additional studies, including immunohistochemical staining and limited molecular genetic analysis, at Massachusetts General Hospital (MGH).

ASSOCIATED CONTENT

Appendix

Author affiliations and support information (if applicable) appear at the end of this article.

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CONTEXT

Key Objective

What is the diagnostic accuracy of newly diagnosed lymphoma cases in a resource-constrained sub-Saharan African country, using Botswana as an example?

Knowledge Generated

Using a tertiary US teaching hospital as the gold standard for diagnostic accuracy, nearly one third of all newly diagnosed cases in Botswana were reclassified. Receipt of a discordant diagnosis was associated with substantially decreased survival.

Relevance

Limitations in pathologic diagnosis contribute to increased mortality from potentially curable lymphomas. Continuing education, partnerships, and novel technologies have potential to reduce mortality.

As a secondary objective, we sought to understand if diagnostic inaccuracy affects overall survival (OS).

METHODS

Patient Cohort

Samples were obtained from patients age 18 years or older with a new diagnosis of lymphoma between 2010 and 2016, treated at three tertiary hospitals in Botswana (Princess Marina Hospital, Gaborone Private Hospital, and Nyangabgwe Referral Hospital), and enrolled in the prospective Thabatse cancer cohort.[7,8] Demographic details, medical history, clinical presentation, socioeconomic variables, prior workup, comorbidities, and HIV status were obtained from medical records and patient interviews. Patients with unknown HIV status underwent diagnostic testing before enrollment. Lymphomas were classified according to the 2017 WHO Classification.[9] To aid in assessing potential clinical implications of discordant pathologic diagnosis, lymphomas were divided into CHL and non-Hodgkin lymphomas (NHLs). There were no cases of nodular lymphocyte predominant HL. NHLs were further categorized according to clinical aggressiveness, with DLBCL, BL, plasmablastic lymphoma (PBL), mantle cell lymphoma, and peripheral T-cell lymphoma (PTCL) considered to be aggressive subtypes requiring more advanced therapeutic approaches over those used for indolent NHL. Clinical staging was based on clinical examination, chest radiographs, and ultrasound imaging only as more advanced staging modalities such as computed tomography, positron emission tomography, or cerebrospinal fluid analysis were not available for most patients. After enrollment, patients were contacted quarterly by telephone, during clinic visits or home visit for 5 years. In the event of a patient's death, the official cause was recorded from the death certificate, with additional context provided by family members and health care workers. The Institutional Review Board of all participating sites approved this study.

Pathologic Diagnosis in Botswana and at MGH

Before study enrollment, all cases underwent histologic confirmation at the Botswana National Health Laboratory. Before March 2013, lymphoma diagnosis was made exclusively by review of hematoxylin and eosin (H&E) stains. A basic immunohistochemical lymphoma panel was introduced in March 2013 that included CD45 (leukocyte common antigen), CD3 (pan T-cell antigen), and CD20 (mature B-cell antigen). Fluorescence in situ hybridization (FISH) and flow cytometry were not available during the study interval. Throughout the study period, certain cases, at the discretion of the local pathologists, were sent to South Africa for consultation, where more extensive immunohistochemistry (IHC) was available and performed, and a diagnosis rendered by the consulting pathologist.

Secondary pathologic review with additional staining was performed by a board-certified hematopathologist (A.R.S.) at MGH, USA, and was considered the gold standard. All cases were sent to MGH with an anonymized copy of the original pathology report from Botswana, the consulting pathologist's report if the case had been sent for consultation to South Africa, and a minimum of one representative formalin-fixed paraffin-embedded tissue block. Each specimen was initially evaluated using an H&E stain cut from the formalin-fixed paraffin-embedded tissue block and stained according to the algorithm outlined in Appendix Table A1.

Statistical Analysis

Baseline characteristics were stratified by comparing pathologic diagnoses in Botswana with diagnoses at MGH (ie, discordant *v* concordant pathology subgroups). Categorical variables were compared with Fisher's exact test, whereas age, the only continuous variable in this report, was compared with the Student's *t*-test. Both the association of different variables with discordant pathologic diagnosis and the association of discordant pathology with OS were assessed using a stratified Cox proportional hazard model, with results matched by CHL versus NHL. In either case, multivariate models were created using variables from the univariate analysis.

In comparing survival outcomes between those with concordant pathologic diagnoses and those without (the discordant group), OS was calculated as time from date of

diagnosis to death or censoring date and univariate analyses were performed via the Kaplan-Meier method and log-rank test. Statistical analyses were performed using SAS version 9.4 (SAS Institute Inc, Cary, NC). All statistical tests used two-sided P values, with $P < .05$ considered to be statistically significant.

RESULTS

Patient Characteristics

There were 70 patient samples included in this study. In the entire cohort, 20 patients (29%) had a discordant diagnosis after review at MGH. Appendix Table A2 shows baseline characteristics stratified by diagnostic discordance. Across all covariates, the two groups were evenly balanced with no significant differences. After evaluation of all cases, secondary pathologic review did not establish a specific diagnosis in three cases: one case was a poorly differentiated nonhematopoietic malignant neoplasm of uncertain lineage not further classifiable; in two cases, definitive classification required clinical information that was not available, including one case of a plasmacytic neoplasm with a differential diagnosis of plasma cell (multiple) myeloma versus PBL and a second case of an atypical cutaneous T-cell infiltrate with a differential diagnosis of drug reaction versus cutaneous T-cell lymphoma. Among the remaining 67 confirmed lymphoma cases, 20 were diagnosed as CHL and 47 as NHL after secondary review at MGH. Notably, 11 cases from this study were evaluated with additional staining in neighboring South Africa and among these, two cases (both BL) had a discordant diagnosis after MGH review.

Comparison of Diagnostic Accuracy and Viral Association

Table 1 shows the original diagnosis in Botswana, the final diagnosis after secondary review across the 20 samples that were reclassified, and the treatment received by each of these reclassified cases, where treatment data were available. All 20 cases diagnosed as CHL at MGH had been previously diagnosed as such in Botswana. However, in eight additional cases diagnosed as CHL in Botswana, this diagnosis was not confirmed upon secondary pathology review: seven of these eight cases were reclassified as subtypes of NHL (Fig 1), whereas one case was diagnosed as an unclassifiable malignant neoplasm. Among the remaining 40 confirmed NHL cases, 11 were reclassified as a different NHL subtype, whereas one case initially diagnosed as a reactive lymph node in a patient clinically

TABLE 1. Details of the 20 Reclassified Cases After Secondary Review at MGH

No.	Original diagnosis in Botswana	Diagnosis After MGH Review	Treatment Received by Patients
		CHL subtypes	
3	Mixed cellularity	DLBCL	1. Bleomycin/doxorubicin/etoposide/vincristine 2. BEACOPP 3. No therapy data
1	Unspecified subtype	DLBCL	Bleomycin/doxorubicin/vinblastine
1	Unspecified subtype	Grade 1-2 follicular lymphoma	ABVD
1	Mixed cellularity	ALK-negative anaplastic large-cell lymphoma	BEACOPP
1	Lymphocyte-rich	Grade 3B follicular lymphoma	No therapy data
1	Nodular sclerosis	Poorly differentiated nonhematologic neoplasm	ABVD
		NHL subtypes	
1	DLBCL	SLL	No therapy data
3	DLBCL	BL	1. CHOP 2. No therapy data 3. No therapy data
1	DLBCL	PTCL, NOS	R-CHOP
3	DLBCL	PBL	1. CHOP 2. No therapy data 3. No therapy data
1	Mantle cell lymphoma	SLL	CHOP
1	Reactive lymphoid hyperplasia	PTCL, NOS	Doxorubicin/vincristine/prednisone
1	PBL	DLBCL	No therapy data
1	PBL	Multiple myeloma (plasma cell myeloma)	No therapy data

NOTE. Results are shown for CHL and NHL subtypes, along with the treatment received by the corresponding patient, where available.

Abbreviations: ABVD, doxorubicin, bleomycin, vinblastine, and dexamethasone; BEACOPP, bleomycin/etoposide/doxorubicin/cyclophosphamide/oncovin (vincristine)/procarbazine; BL, Burkitt lymphoma; CHL, classic Hodgkin lymphoma; CHOP, cyclophosphamide/doxorubicin (doxorubicin)/vincristine/prednisone; DLBCL, diffuse large B-cell lymphoma; MGH, Massachusetts General Hospital; NHL, non-Hodgkin lymphoma; NOS, not otherwise specified; PBL, plasmablastic lymphoma; PTCL, peripheral T-cell lymphoma; R, Rituximab; SLL, small lymphocytic lymphoma.

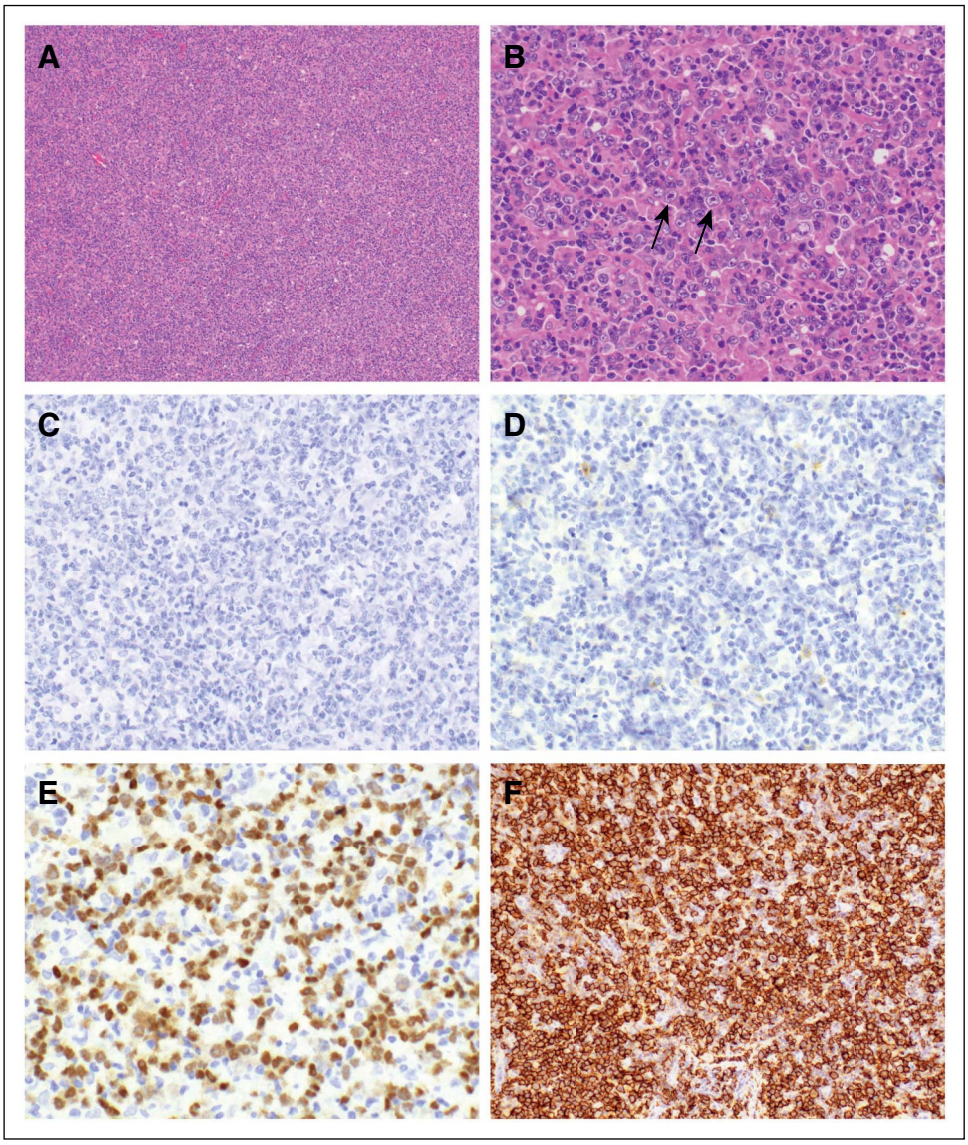

FIG 1. An example of DLBCL initially classified as CHL, mixed cellularity type. (A) H&E-stained sections demonstrated a diffuse inflammatory cell infiltrate with effacement of the underlying nodal architecture. (B) On higher magnification, the infiltrate was composed of large atypical mononuclear cells with prominent macronucleoli (arrows) in a background of small lymphocytes. Although no classic binucleate Reed-Sternberg cells were seen, the resemblance of the large atypical cells to mononuclear Hodgkin cell variants and relatively frequent background non-neoplastic inflammatory cells led to a diagnosis of CHL on the basis of routine H&E stains. (C-E) Immunohistochemical stains demonstrated that the large atypical cells were negative for (C) CD30 and (D) CD15, but stained positively for (E) PAX5, a pan B-cell marker. (F) An additional stain for CD20 was strongly positive, confirming the diagnosis of DLBCL. CHL, classic Hodgkin lymphoma; DLBCL, diffuse large B-cell lymphoma; H&E, hematoxylin and eosin.

suspected to have lymphoma was reclassified as PTCL, not otherwise specified (NOS). Although many of the reclassified diagnoses involved a change that did not alter the category of clinical aggressiveness (eg, DLBCL reclassified as BL; PBL; or PTCL, NOS), there were four cases where the clinical implications of the revised diagnosis were more profound, including one DLBCL reclassified as chronic lymphocytic leukemia (CLL; Fig 2), one mantle cell lymphoma reclassified as CLL, one PBL reclassified as multiple myeloma, and one reactive lymph node reclassified as PTCL, NOS.

Table 2 shows the HIV and Epstein-Barr virus (EBV) viral associations across all CHL and NHL subtypes. Most CHLs were EBV-associated (15 of 20, 75%) and slightly fewer were HIV-associated (12 of 20, 60%). Among NHL cases, DLBCL was the most common aggressive B-cell lymphoma with 28 cases, 68% of which were associated with HIV, but only 11% of which were confirmed to be EBV-associated. Among other aggressive B-cell lymphomas with an established HIV association, all five PBLs were HIV- and EBV-associated. By contrast, all three BLs were HIV-associated, but none were EBV-associated. Among the remaining NHLs, two PTCL, NOS; two CLL; and one low-grade follicular lymphoma were associated with HIV, none of which were EBV-associated.

Survival Outcome

With a median follow-up of 58 months (interquartile range: 14-60 months) among survivors, there were 14 deaths among the 20 patients with discordant pathology and 20 deaths within the subgroup of 48 patients with concordant pathology. Figure 3 illustrates OS stratified by pathologic diagnosis concordance across all patients. The unadjusted 2-year OS rate was significantly higher for those with concordant diagnoses (74.5% *v* 52.6%), and this difference persisted at 5 years (60.1% *v* 26.3%), log-rank *P* value = .0066.

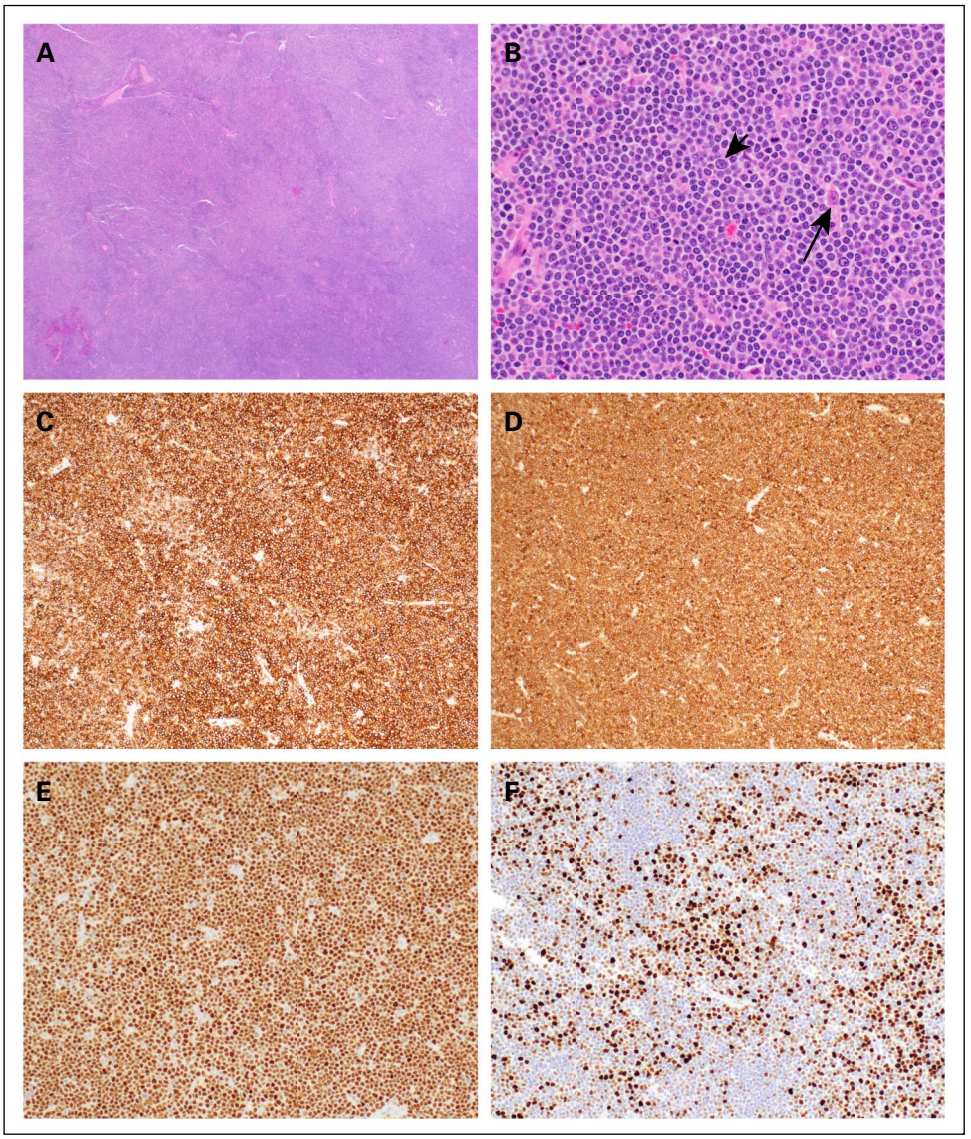

FIG 2. An example of SLL initially classified as DLBCL. (A) H&E-stained sections demonstrated diffuse nodal architectural effacement by an infiltrate containing vaguely nodular pale-staining areas, consistent with proliferation centers. (B) On higher magnification, the lymphocytes appeared monomorphous with clumped chromatin and occasional small nucleoli. The cellular monomorphism and extent of nodal architectural effacement prompted a diagnosis of DLBCL on the basis of routine H&E stains. Further evaluation showed that most of the lymphocytes were smaller than endothelial cells (arrow) with only occasional large cells seen (arrowhead). (C-E) The lymphoma cells were diffusely positive for (C) CD20, confirming B-cell lineage, with aberrant expression of the T-cell markers, (D) CD5 and (E) LEF1, supporting a diagnosis of SLL. (F) A stain for Ki67 was not uniformly elevated as would be expected for DLBCL, but showed a variable proliferation index, with higher staining within proliferation centers but relatively low staining outside of proliferation centers, in line with SLL. DLBCL, diffuse large B-cell lymphoma; H&E, hematoxylin and eosin; SLL, small lymphocytic lymphoma.

Predictors of Discordant Pathology and Survival

The univariate and multivariate association between covariates and OS is shown in Table 3, wherein the covariates are stratified and matched by subtype (ie, CHL *v* NHL) to account for differences in baseline hazard proportion. Discordant pathologic diagnosis was associated with 2.7 times increased risk of death, and this association was independent of any other factors (adjusted hazard ratio [AHR] 2.733; 95% CI, 1.102 to 6.755; $P = .03$). Clinical aggressiveness, as defined in the Methods section, was associated with worse OS on univariate analysis (AHR 1.975; 95% CI, 0.988 to 3.948; $P = .0542$) but not on multivariate analysis (AHR 1.685; 95% CI, 0.703 to 4.039; $P = .2421$). Older age and poor performance status were each associated with increased risk of death on univariate analysis, with differences no longer statistically significant after adjusting for other factors. Similarly, HIV disease was not independently associated with worse OS after adjusting for potential confounding variables. On sensitivity analysis, EBV status was not associated with OS on univariate analysis. In determining predictors of discordant pathologic diagnosis on univariate and multivariate analyses, no demographic, patient-specific socioeconomic factors or disease attributes were associated with diagnostic discordance (Table 4). Neither was the presence of HIV associated with discordant diagnosis.

DISCUSSION

In this single prospective observational cohort of patients presenting with a new diagnosis of lymphoma at three tertiary hospitals in Botswana, the overall pathologic reclassification rate was 29%. Although CHL cases were correctly classified, a significant proportion of NHL cases were incorrectly classified, including 21% of DLBCLs, which bears significant implications on recommended therapy. None of the three BLs and only 2 of 5 PBLs were

TABLE 2. Subtype Distribution of the 20 Confirmed CHL and 47 Confirmed NHL Cases With Their Corresponding Viral Association With EBV and HIV

Subtype	No. (%)	EBV+	EBV–	EBV Unknown	HIV+	HIV–	HIV Unknown
CHL							
Mixed cellularity	11 (55)	8	2	1	7	4	0
Nodular sclerosis	6 (30)	4	1	1	4	2	0
Lymphocyte-rich	2 (10)	2	0	0	1	1	0
Lymphocyte-depleted	1 (5)	1	0	0	0	1	0
NHL							
DLBCL	28 (60)	3	15	10	19	9	0
PBL	5 (11)	5	0	0	5	0	0
BL[a]	3 (6)	0	2	1	3	0	0
Mantle cell lymphoma	1 (2)	0	0	1	0	1	0
SLL	4 (9)	0	0	4	2	2	0
Low-grade follicular lymphoma	2 (4)	0	1	1	1	0	1
ALK-negative anaplastic large-cell lymphoma	2 (4)	0	2	0	0	1	1
PTCL, NOS	2 (4)	0	1	1	2	0	0

Abbreviations: BL, Burkitt lymphoma; CHL, classic Hodgkin lymphoma; DLBCL, diffuse large B-cell lymphoma; EBV, Epstein-Barr virus; FISH, fluorescence in situ hybridization; NHL, non-Hodgkin lymphoma; NOS, not otherwise specified; PBL, plasmablastic lymphoma; PTCL, peripheral T-cell lymphoma; SLL, small lymphocytic lymphoma.

[a]*MYC* rearrangement detected by FISH in two of the three evaluable cases.

accurately classified locally. Patients with discordant pathologic diagnoses had worse 5-year OS compared with those with concordant results, with a nearly three-fold increased risk of dying. There were no patient-specific, disease-specific, or socioeconomic variables associated with discordant pathology. Patients with HIV had a lower risk of diagnostic discordance although this result was not significant after adjusting for other covariates (Table 4).

To our knowledge, this series is the first to quantify the impact of inaccurate diagnosis among patients with lymphoma from sub-Saharan Africa (SSA). As distinct regimens have proven to be most effective for common lymphoma subtypes, including R-CHOP (rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone) for DLBCL, R-EPOCH (R-CHOP plus etoposide) for adult BL, and ABVD (doxorubicin, bleomycin, vinblastine, and dexamethasone) for CHL,

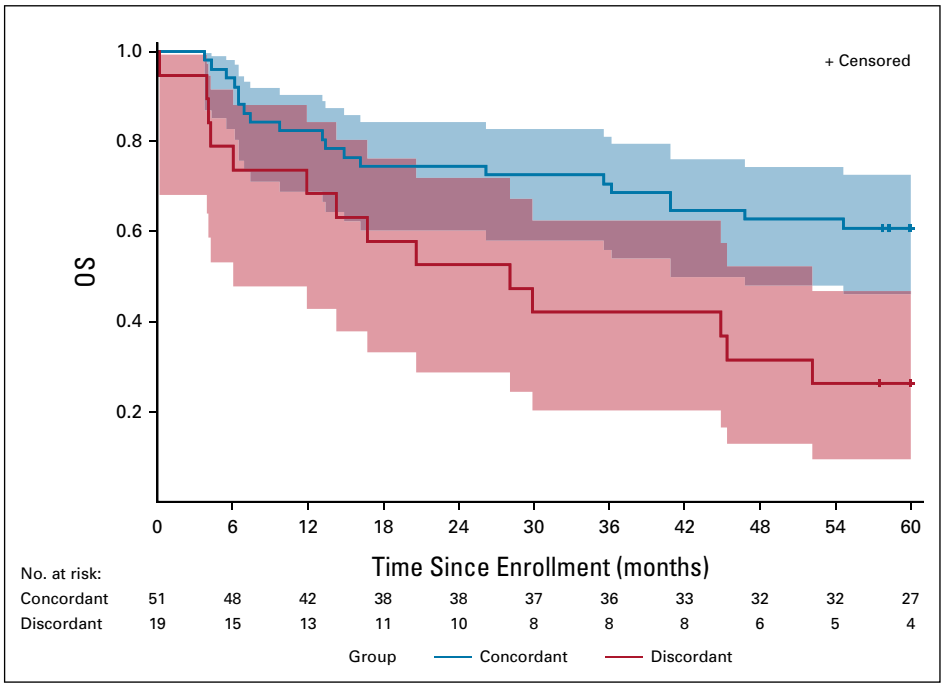

FIG 3. Kaplan-Meier survival estimate for patients with concordant versus discordant pathologic diagnosis after MGH review with number of patients at risk and 95% confidence limits. MGH, Massachusetts General Hospital; OS, overall survival.

TABLE 3. Association Between Various Patient or Disease Characteristics and Overall Survival

	Univariate Analysis			Multivariate Analysis		
Variable	**Hazard Ratio**	**95% CI**	***P***	**Hazard Ratio**	**95% CI**	***P***
Pathologic diagnosis concordance after review at MGH						
Concordant	1			1		
Discordant	2.503	1.260 to 4.973	.0088	2.733	1.102 to 6.775	.0300
Age	1.04	1.013 to 1.062	.0023	1.028	0.997 to 1.060	.0722
Sex						
Male	1			1		
Female	1.204	0.614 to 2.360	.5883	1.318	0.552 to 3.148	.5344
ECOG performance status						
0-1	1			1		
2-4	2.509	1.279 to 4.937	.0078	1.601	0.748 to 3.431	.2257
HIV status						
HIV–	1			1		
HIV+	0.565	0.285 to 1.121	.1026	0.695	0.306 to 1.580	.3849
Lymphoma stage						
I	1			1		
II	0.906	0.234 to 3.507	.8869	1.158	0.279 to 4.797	.8402
III	1.982	0.718 to 5.471	.1866	2.592	0.832 to 8.077	.1004
IV	1.783	0.664 to 4.792	.2513	1.183	0.408 to 3.424	.7572
Unknown or missing	4.126	1.438 to 11.838	.0084	4.604	1.296 to 16.352	.0182
Aggressive lymphoma						
No	1			1		
Yes	1.975	0.988 to 3.948	.0542	1.685	0.703 to 4.039	.2421

NOTE. Univariate and multivariate models are shown.
Abbreviations: ECOG, Eastern Cooperative Oncology Group measure of performance status; MGH, Massachusetts General Hospital.

it appears likely that failure to correctly identify lymphoma subtype results in significantly increased patient mortality. In particular, omission of rituximab in treating NHL would have a significant negative effect.

The high reclassification rate in this series is consistent with reports from other parts of SSA where lymphoma diagnostic error rates, regardless of subtype, have been reported to range between 22% and 38%.[10-12] Multiple factors likely contribute to this lower rate of pathologic diagnostic accuracy in resource-limited settings compared with developed countries.

Lack of relatively basic diagnostic techniques such as IHC has been described as the biggest barrier to achieving diagnostic accuracy.[10] For example, almost none of the cases of CHL in this series were stained with IHC, which is helpful for confirming the diagnosis and excluding NHL entities in the differential diagnosis. Similarly, none of the three BL cases in this series were accurately classified locally, including the two cases sent in consultation to South Africa. Under guidance of the International Network for Cancer Treatment and Research group, in 2011, a group of African pathologists and oncologists sought to understand the state of pathologic infrastructure at major hospitals in several African countries with the goal of identifying capacity-building measures.[10] Many centers in Africa rely primarily on morphologic examination on routine stains (eg, H&E and Papanicolaou) in diagnosing new cases of lymphoma. A few countries still rely on clinical symptoms and basic imaging alone in diagnosing new cancers. For example, a Malawi national cancer registry reported only 18% of cancer diagnoses as having pathologic confirmation.[13] A Ugandan series of 19 BLs that underwent secondary pathology review in the Netherlands showed a diagnostic concordance of only 32%.[12] In the absence of good morphology, standard IHC panels, and FISH for *MYC* rearrangement, BL is difficult to distinguish from DLBCL,[14-17] making such cases difficult to classify in resource-limited countries.

In countries that do not routinely obtain biopsy tissue, indistinguishable clinical symptoms between lymphoma and other endemic or coinfectious diseases, such as tuberculosis or HIV, is another leading cause of lymphoma underdiagnosis.[18-21] However, all cases in our cohort underwent tissue diagnosis. There was no significant association between HIV status and pathologic discordance,

TABLE 4. Assessing Predictors of Discordant Pathologic Diagnosis

	Univariate Analysis			Multivariate Analysis		
Variable	**Hazard Ratio**	**95% CI**	***P***	**Hazard Ratio**	**95% CI**	***P***
Age	1.02	0.984 to 1.086	.2849	1.003	0.963 to 1.046	.8792
Sex						
Male	1			1		
Female	0.909	0.365 to 2.264	.8382	0.483	0.162 to 1.443	.2456
ECOG performance status						
0-1	1			1		
2-4	1.865	0.741 to 4.696	.1857	1.883	0.654 to 5.137	.2489
HIV status						
HIV–	1			1		
HIV+	0.424	0.157 to 1.146	.0908	0.645	0.209 to 1.993	.4464
Lymphoma stage						
I	1			1		
II	0.678	0.137 to 3.364	.6349	0.433	0.083 to 2.258	.3205
III	1.47	0.448 to 4.822	.5252	1.803	0.485 to 6.696	.3787
IV	0.98	0.276 to 3.479	.9751	0.581	0.151 to 2.239	.4303
Unknown or missing	1.472	0.295 to 7.349	.6377	0.638	0.100 to 4.050	.6336
Aggressive lymphoma						
No	1			1		
Yes	1.41	0.572 to 3.477	.4588	0.564	0.202 to 1.572	.2732

NOTE. Univariate and multivariate models examining the association between various patient or disease characteristics and probability of discordant pathology are shown.

Abbreviation: ECOG, Eastern Cooperative Oncology Group measure of performance status.

which correlates with reports by two previous studies showing no clinical differences in lymphoma outcomes between patients with and without HIV.[8,22] As expected, certain diagnoses, including BL, PBL, CHL, and DLBCL, showed strong associations with HIV; subsets of these cases were also EBV-associated. Small numbers of cases in

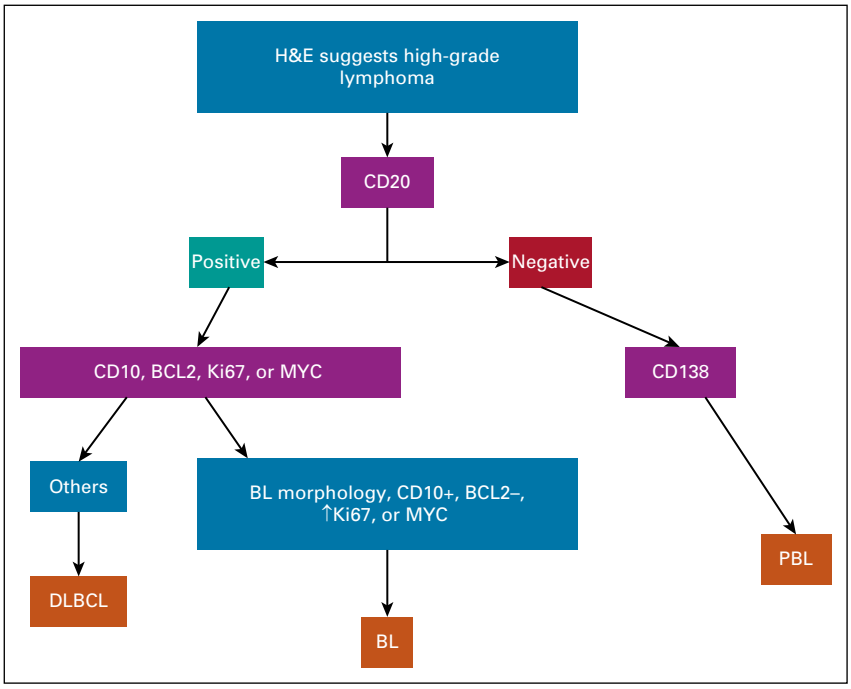

FIG 4. Proposed diagnostic algorithm for high-grade B-cell lymphomas. When routine H&E examination suggests high-grade lymphoma, CD20 is initially performed to support B-cell lineage. DLBCL and BL can then be distinguished on the basis of a staining panel that includes CD10, BCL2, and either Ki67 or MYC. Cases that are negative for CD20 can be stained for CD138 to support a diagnosis of PBL. BL, Burkitt lymphoma; DLBCL, diffuse large B-cell lymphoma; H&E, hematoxylin and eosin; PBL, plasmablastic lymphoma. Adapted from the study by Sayed et al.[29]

the other lymphoma categories make it difficult to draw conclusions regarding HIV association; however, both cases of PTCL, NOS were HIV-associated, representing 50% of all PTCLs. Although most HIV-associated lymphomas are of B-cell lineage, US cancer and AIDS data registries show that patients with HIV have a 15-fold greater relative risk of developing PTCL compared with the general population.[23]

Other possible reasons for the lower rate of pathologic accuracy in diagnosing lymphoma include suboptimal histology, use of outdated diagnostic criteria in lymphoma classification, and lack of advanced training for pathologists and laboratory technologists.[10,11,24,25] The number of pathologists in low-resource areas is about 10% the capacity of that in developed countries, so this mismatch between specimen volume and human capital limits opportunities for subspecialization and leads to specimen backlogs, which may result in perceived lack of relevance of pathology since diagnoses are rendered after therapeutic decisions are undertaken.[26]. The College of Pathologists of East, Central and Southern Africa (COPECSA) is an internationally recognized body recently established to ensure quality training programs and professional development opportunities for junior pathologists in the region.[24,27] Furthermore, evolving technologies, such as IHC and FISH, necessitate specialized training for laboratory staff and equipment and supply chain optimization for reagent procurement, which can be bolstered by specific laboratory improvement initiatives to address these challenges.[28]

An additional key solution to overcome challenges of lymphoma diagnosis in SSA is establishing standard diagnostic algorithms. For instance, a previous study demonstrated that, even in the absence of FISH, a diagnostic algorithm of four IHC stains can be used to accurately distinguish between DLBCL and BL, with additional staining in CD20-negative cases to help support a diagnosis of PBL (Fig 4).[29] This limited staining panel would have identified all cases of BL and PBL in our series that were originally misdiagnosed as DLBCL without the need for advanced techniques like FISH or Epstein-Barr virus–encoded small RNA in situ hybridization. Similarly, our findings suggest that a basic IHC panel of CD30 and PAX5 can be performed on cases of suspected CHL to confirm the diagnosis (Fig 1). These two stains alone would have avoided a misdiagnosis of CHL in all eight cases in our series that were ultimately diagnosed as B-cell NHL, CD30+ PTCL, or a nonhematologic malignancy.

In addition to solutions proposed above, telepathology conferences can foster exchange of expertise and exposure to challenging diagnostic entities uncommon to low-resource settings, eg, low-grade lymphoma.[10,25,30-35] Pathology-based research through collaborations between high- and low-resource countries is another mechanism to facilitate knowledge transfer.[36] These partnerships may lead to establishing centers of diagnostic excellence within SSA with the capacity to address regional needs through a hub-and-spoke organizational approach.[37] Importantly, funding such partnerships through nongovernmental organizations or ministries of health increases access of quality pathology services to more patients than when pathology laboratories are purely privately owned. Therefore, a sustainable long-term solution would be to develop regional centers of diagnostic expertise rather than sending consultations to distant experts in the United States or Europe. Similar studies show that the average turnaround time for distant expert consultation ranges from 7 to 14 days, but establishing a robust mechanism for regional pathologic consultations can decrease this to five days with significantly lower costs.[28] Thus, adoption of regional centers of expertise would decrease turnaround time to diagnosis, reduce overall costs, and strengthen regional collaborations among health care providers in SSA, thereby fostering a sense of community among pathology and laboratory personnel in the region.

This study has several limitations. The relatively small size of the cohort limits the power of the statistical analysis. Patients were diagnosed with heterogenous disease entities with varying baseline risk factors, natural history, and prognostic outcomes. We attempted to overcome this heterogeneity by performing the stratified Cox proportional hazard model in the adjusted model and matching results by CHL versus NHL. However, the potentially worse OS resulting from inherently difficult diagnoses, such as BL, cannot be captured by multivariate analysis. The analysis of this data was done retrospectively; however, we aimed to minimize selection bias by limiting analysis to a population followed prospectively in a registry cohort. Although clinicopathologic data were obtained from three tertiary hospitals, pathologic diagnosis was performed at one central facility in Botswana, potentially threatening the generalizability of our findings to other laboratories in low-resource areas.

Despite these limitations, we believe that this study underscores the importance of access to IHC, a relatively basic diagnostic tool in high-income countries, and demonstrates the potentially serious impact of discordant pathologic diagnosis on treatment decisions and patient mortality.

AFFILIATIONS

[1]Harvard Radiation Oncology Program, Harvard Medical School, Boston, MA
[2]Department of Radiation Oncology, Massachusetts General Hospital, Boston, MA
[3]University of Botswana, Gaborone, Botswana
[4]Ministry of Health, Gaborone, Botswana
[5]Botswana Harvard AIDS Institute, Gaborone, Botswana
[6]Massachusetts General Hospital Cancer Center, Boston, MA
[7]Division of Infectious Diseases, Department of Medicine, Brigham and Women's Hospital, Boston, MA
[8]Department of Pathology, Massachusetts General Hospital, Boston, MA

CORRESPONDING AUTHOR

Fallon E. Chipidza, MD, Department of Radiation Oncology, Massachusetts General Hospital, 55 Fruit St, Boston, MA 02114; e-mail: fchipidza@dfci.harvard.edu.

EQUAL CONTRIBUTION

S.L.D.-P. and A.R.S. contributed equally to this work.

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AUTHOR CONTRIBUTIONS

Conception and design: Fallon E. Chipidza, Jason A. Efstathiou, Bruce A. Chabner, Scott L. Dryden-Peterson, Aliyah R. Sohani
Administrative support: Jason A. Efstathiou, Bruce A. Chabner, , Aliyah R. Sohani
Provision of study materials or patients: Mukendi K. A. Kayembe, Isaac Nkele, , Aliyah R. Sohani
Collection and assembly of data: Fallon E. Chipidza, Mukendi K. A. Kayembe, Isaac Nkele, Scott L. Dryden-Peterson, Aliyah R. Sohani
Data analysis and interpretation: Fallon E. Chipidza, Jason A. Efstathiou, Bruce A. Chabner, Jeremy Abramson, Scott L. Dryden-Peterson, Aliyah R. Sohani
Manuscript writing: All authors
Final approval of manuscript: All authors
Accountable for all aspects of the work: All authors

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Consulting or Advisory Role: Blue Earth Diagnostics, AstraZeneca, Boston Scientific, Roivant, Merck, Myovant Sciences

Bruce A. Chabner
Stock and Other Ownership Interests: Alnylam, Loxo, PharmaMar, Blueprint Medicines, GlaxoSmithKline, Biomarin, Seattle Genetics, SpringWorks Therapeutics, Constellation Pharmaceuticals
Honoraria: Bristol Myers Squibb
Consulting or Advisory Role: PharmaMar, EMD Serono, Cyteir, Chugai Pharma
Expert Testimony: Lilly
Travel, Accommodations, Expenses: PharmaMar

Jeremy Abramson
Consulting or Advisory Role: Celgene, Novartis, AbbVie, Kite, a Gilead company, Genentech, EMD Serono, MorphoSys, Alimera Sciences, Karyopharm Therapeutics, Bristol Myers Squibb, C4 Therapeutics, BeiGene, AstraZeneca, Incyte, Bluebird Bio, Kymera, Epizyme, Genmab, MorphoSys, MustangBio, Ono Pharmaceutical, Regeneron
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Aliyah R. Sohani
Consulting or Advisory Role: AbbVie, Mersana
Expert Testimony: Levin Papantonio

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APPENDIX

TABLE A1. Diagnostic Workup of Cases Sent for Secondary Pathology Review at Massachusetts General Hospital on the Basis of Evaluation of Initial Hematoxylin and Eosin–Stained Slide(s)

Suspected Diagnosis	Immunohistochemical Stains and Other Studies Performed
Large-cell or high-grade lymphoma (including BL and cases with plasmacytic or plasmablastic differentiation)	Initial: CD20, CD5, CD10, BCL6, MUM1, BCL2, MYC, Ki67, EBER ISH CD20-negative, CD5-positive cases: T-cell antigens, CD30, ALK1, TdT
BL	*MYC* FISH
Plasmacytic or plasmablastic differentiation	CD138, kappa light chain, lambda light chain, HHV-8 LANA; CD56 and ALK1 if sufficient tissue
CHL	CD30, CD15, PAX5/BSAP, EBER ISH
Small-cell or low-grade lymphoma	Initial: CD20, CD5, Ki67 CD5-positive cases: cyclin D1, LEF1 CD5-negative cases: CD10, BCL6, BCL2, CD21

Abbreviations: ALK1, anaplastic lymphoma kinase-1; BL, Burkitt lymphoma; BSAP, B-cell–specific activator protein; CHL, classic Hodgkin lymphoma; MUM1, multiple myeloma oncogene-1; EBER ISH, Epstein-Barr virus–encoded small RNA in situ hybridization; FISH, fluorescence in situ hybridization; HHV-8 LANA, human herpes virus-8 latency-associated nuclear antigen; LEF1, lymphoid enhancer binding factor-1; TdT, terminal deoxynucleotidyl transferase.

TABLE A2. Baseline Patient Characteristics Stratified by Pathologic Diagnosis Concordance After Secondary Review With Additional Staining at MGH

Variable	Discordant Diagnosis (n = 19)	Concordant Diagnosis (n = 51)[a]	*P*
Median age, years (IQR)	43 (32-58)	40 (35-51)	.6818
Sex, No. (%)			.9999
Male	11 (58)	27 (53)	
Female	8 (42)	22 (43)	
ECOG performance status, No. (%)			.8854
0-1	11 (58)	31 (61)	
2-4	8 (42)	18 (35)	
HIV status, No. (%)			.8804
HIV+	12 (63)	34 (67)	
HIV–	7 (37)	15 (29)	
Lymphoma stage, No. (%)			.9121
I	6 (32)	13 (25)	
II	2 (11)	8 (16)	
III	5 (26)	10 (20)	
IV	4 (21)	11 (22)	
Unknown	2 (11)	9 (18)	
Aggressive lymphoma,[b] No. (%)			.7900
Yes	10 (53)	24 (47)	
No	9 (47)	27 (53)	
Education level, No. (%)			.8804
Primary school only	7 (37)	15 (29)	
High school or higher	12 (63)	34 (67)	
Marital status, No. (%)			.8798
Married	5 (26)	16 (32)	
Unmarried	14 (74)	33 (65)	
Rural residence, No. (%)			.4288
Yes	11 (58)	35 (69)	
No	8 (42)	14 (27)	
Household income, No. (%)			.6954
< USD $50/month	7 (37)	23 (45)	
> USD $50/month	12 (63)	26 (51)	

Abbreviations: IQR, interquartile range; MGH, Massachusetts General Hospital; USD, US dollars.

[a]Only basic diagnosis details and pathologic concordance data were available for two of the patients in this cohort. Their data were included in constructing Table 1, but missing attributes for some of the variables resulted in some of the column totals not adding up to 100%.

[b]Aggressive lymphoma determination on the basis of reclassified MGH diagnosis.

