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Assessment of the Use of Available Resources for Diagnosing Diffuse Large B-Cell Lymphoma in an HIV-Prevalent Setting

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ABSTRACT

Background: Limited availability of diagnostic tests in low-resource settings hampers the diagnosis and classification of diffuse large B-cell lymphoma (DLBCL). A study was performed to assess the use of resources for classifying DLBCL in South Africa (SA) using 'essential' and 'desirable' investigations as per published guidelines.

Methods: A record review was performed of 74 patients newly diagnosed with DLBCL by tissue biopsy at the National Health Laboratory Service (NHLS) in Johannesburg between 1 January 2019 and 31 December 2022. The immunohistochemistry (IHC) and/or molecular work-up performed for the primary diagnosis of DLBCL and the associated costs were recorded.

Results: The primary diagnosis of DLBCL was based on 34 (45.9%) nodal and 40 (54.1%) extra-nodal biopsy sections. Overall, 60 (81.1%) were from participants living with human immunodeficiency virus (HIV) infection. 'Essential' IHC for CD3, CD10, CD20, Ki-67, BCL-2, BCL-6, MUM-1 and 'desirable' fluorescence in situ hybridisation (FISH) for *MYC* gene rearrangement were most requested for diagnosis. 'Essential' IHC for c-MYC was not performed because of non-availability of the testing. The 'essential' IHC was diagnostic in 97.3%. 'Desirable' FISH for *MYC* rearrangement was done in 56 (79.7%) cases, with additional FISH for *BCL2* and *BCL6* rearrangement performed in cases positive for *MYC* rearrangement. The average cost of diagnosis at the NHLS was half that of the recommended diagnostic testing.

Conclusion: The advocated 'essential' investigations, in addition to 'desirable' tests where necessary, enabled the accurate and cost-effective diagnosis of DLBCL in SA and are recommended for other parts of the world with limited resources.

1 | Introduction

Non-Hodgkin lymphoma (NHL) is a heterogeneous group of clonal lymphoid neoplasms of B cell, T cell and natural killer lymphocytes [1]. Worldwide, according to the Global Cancer Observatory statistics, there were 553 010 new diagnoses and 250 475 deaths related to NHL in 2022 [2]. In South Africa, a middle-income country, NHL has been reported amongst the top 10 cancers with a five-year prevalence of 3.6 per 100 000, which corresponded to 3259 new cases (2.9% of all cancers) and 1867 deaths in 2022 [3]. Large B cell lymphoma subtypes, including diffuse large B-cell lymphoma (DLBCL) are more common in Southern Africa, whereas the proportion of low-grade B cell lymphoma subtypes is higher in Western Europe and North America [4].

The high incidence of large B cell lymphoma in Southern Africa has been attributed to infection with Human

Immunodeficiency Virus (HIV). NHL occurs in people living with HIV (PLWH) owing to co-infections with oncogenic viruses and impaired immune surveillance, and has contributed to the increased cancer-related morbidity and mortality in these patients [5]. South Africa has one of the largest HIV epidemics in the world, with approximately 7.6 million PLWH according to the most recent national estimate, with 75% being on antiretroviral therapy (ART) [6]. The incidence and mortality of NHL since the introduction of ART have shown a decline [7]. A study of the South African National Cancer Registry reported a three-fold increased risk of NHL amongst PLWH between 2004 and 2014 as compared to a six-fold increased risk in a pre-ART study from 1995 to 2004 [8, 9]. NHL, however, continues to represent a major source of mortality among PLWH in Africa [10].

The most common NHL subtype in PLWH is DLBCL, which accounts for 38.2% of all NHL in adults in South Africa [3]. The World Health Organization (WHO) Classification of Haematolymphoid Tumours and the International Consensus Classification (ICC) of Mature Lymphoid Neoplasms have contributed to standardising the classification of DLBCL with classification according to morphological, immunophenotypical and molecular characteristics into specific subtypes [1, 11, 12]. Further sub-classification according to the cell-of-origin into germinal centre B-cell (GCB) and non-germinal centre subtypes is recommended for prognostic purposes, with the non-germinal centre subtype reportedly being associated with a poorer prognosis [13]. The cell-of-origin can be determined by gene expression profiling or immunohistochemistry (IHC) staining for CD10, BCL6 and multiple myeloma oncogene 1 (MUM1) with interpretation using the Hans algorithm [14]. Immunohistochemistry alone, however, does not fully capture the genetic diversity of DLBCL, and molecularly defined subtypes of DLBCL with distinct molecular and clinical features are recognised. Various algorithms have been proposed over the years to limit the number of newly diagnosed DLBCL cases that are sent for fluorescence in situ hybridisation (FISH) analysis to reduce the cost of testing. Several investigators have examined overexpression of MYC $\geq 40\%$ and BCL2 $\geq 50\%$ by IHC as a screening tool for *MYC* rearrangements. This approach has been associated with a false negative rate of 10%–30% for *MYC* rearrangement, and as such is not recommended [15, 16]. There is consequently no recommended predictive IHC panel for determining which cases of DLBCL should undergo molecular screening.

The importance of molecular testing in the classification of DLBCL subtypes was acknowledged by the prior 4th edition of the WHO Classification (2017) [11]. The latest 5th edition of the WHO Classification, updated in 2022, has taken into account the limited availability of resources in some settings and has introduced 'essential' and 'desirable' diagnostic criteria for DLBCL [1]. The 5th edition of the WHO Classification considers the assignment of B-cell lineage to a large B-cell lymphoma with a diffuse or vaguely nodular growth pattern and the exclusion of other common, specific entities of large B-cell lymphoma as 'essential', while assessment of the cell-of-origin and the performance of FISH analysis for *MYC* +/− *BCL6* gene rearrangements and genetic testing if relevant are considered 'desirable' [1]. In addition, the 5th edition includes IHC for underlying oncogenic viral infection (Epstein–Barr virus [EBV] and Kaposi's sarcoma-associated herpesvirus [KSHV]) as further 'desirable' work-up in DLBCL arising in immune deficiency/dysregulation [1]. 'Essential' and 'desirable' diagnostic criteria have also been extended to IHC stains. According to the recommendation by Cho, essential IHC includes: CD3, CD10, CD20, Ki-67, BCL-2, BCL-6, MUM-1, c-MYC, EBV-encoded RNA in situ hybridization (EBER ISH) and the desirable include: CD79a, CD30, CD23, ALK and CD138 [17].

In this study, we assessed the use of available diagnostic resources for the diagnosis of DLBCL in South Africa, a resource-limited setting. Moreover, we evaluated the cost implications of performing the full recommended work-up, compared to what is typically done.

2 | Methods

2.1 | Study Design and Population

A retrospective record review was performed on a cohort of newly diagnosed participants with DLBCL by tissue biopsy at the National Health Laboratory Service (NHLS) in Johannesburg, South Africa between 1 January 2019 and 31 December 2022. Participants were enrolled in a previous study entitled 'Evaluation of the association between tumour enrichment with M2-macrophages and survival among South African patients with Diffuse Large B-cell Lymphoma'. Cases with inadequate information or inconclusive histopathological findings ($n = 2$) were excluded. This study was approved by the Human Research Ethics committee of the University of the Witwatersrand (Protocol number: M240438).

2.2 | Data Collection

Participants were identified from a password-protected digital database that was maintained by the supervisor (JV) and included 74 cases of DLBCL diagnosed and classified according to the 4th edition of the WHO. The diagnosis was confirmed in each participant by reviewing the NHLS Laboratory Information System (TrakCare Labs v6.10, InterSystems Corporation, Cambridge, MA, 2012). The primary diagnosis was made based on formalin-fixed paraffin tissue biopsy sections stained with Haematoxylin and Eosin. The cell of origin was determined using the Hans algorithm [14]. 'Essential' IHC included: CD3, CD10, CD20, Ki-67, BCL-2, BCL-6, MUM-1, c-MYC, EBV-encoded RNA in situ hybridization (EBER ISH) and 'desirable' included: CD79a, CD30, CD23, ALK and CD138 (as per Cho) [17].

The following tests performed for the diagnosis of DLBCL were recorded: IHC stains, flow cytometry (FC), cytogenetics, FISH and polymerase chain reaction (PCR) for detection of clonality of immunoglobulin heavy chain (IgH) gene rearrangement. Corresponding HIV status, HIV viral load (VL) and CD4 count, age, ethnicity and gender were collected from the database. The direct costs of the diagnostic and additional investigations were determined according to the current NHLS pricing.

2.3 | Data Analysis

Data were recorded in a Microsoft Excel spreadsheet (Version 16.78) and analysed using Statistica Software version 13.2 (Statistica, Tulsa, USA). Normally distributed continuous data were presented as mean ± standard deviation (SD) and variables with non-Gaussian distribution as median (interquartile range [IQR]). Categorical data were presented as frequencies and percentages. Comparisons for continuous measurements between the study groups subdivided according to HIV negative and PLWH were performed using a parametric unpaired *t*-test or non-parametric Mann–Whitney test, as appropriate. Statistical comparisons were performed using chi-square test or Fisher's exact test when necessary for categorical variables. The significance level was set at $p < 0.05$.

3 | Results

3.1 | Participant Characteristics

A total of 74 participants were included. The demographics and laboratory characteristics of the participants stratified according to HIV status are described in Table 1. The majority were of African ethnicity, in keeping with the demographics of the population. DLBCL was more common amongst males ($n = 43$, 58.1%), with a mean age of 44 ± 12 years. There were 60 (81.1%) PLWH, of which 19 (31.7%) were virologically suppressed. PLWH at diagnosis were significantly younger, at a mean ± SD age of 42 ± 11 years ($p < 0.024$). DLBCL was highest amongst black males ($n = 34$, 79.1%), with a mean age of 44 ± 12 years.

TABLE 1 | Baseline characteristics of participants with DLBCL according to HIV.

	Results			
Characteristic	**Total ($n = 74$)**	**HIV + ($n = 60$)**	**HIV − ($n = 14$)**	***p***
Demographics				
Age (years) at diagnosis, mean ± SD	44.0 ± 13.0	42.0 ± 11.1	51.0 ± 17.9	0.024
Gender				
Male (*n*, %)	43 (58.1)	34 (79.1)	9 (20.9)	0.766
Female (*n*, %)	31 (41.9)	26 (83.9)	5 (16.1)	
Ethnicity				
African (*n*, %)	43 (58.1)	34 (79.1)	9 (20.9)	0.399
Other (*n*, %)	2 (2.7)	1 (50.0)	1 (50.0)	
Missing (*n*, %)	29 (39.2)	25 (86.2)	4 (13.8)	
Laboratory characteristics				
CD4 cell count ($\times 10^6$/L), median [IQR]		143[69.5–287.3]		
$< 200 \times 10^6$/L, (*n*, %)		42 (70.0)		
$\geq 200 \times 10^6$/L, (*n*, %)		18 (30.0)		
HIV viral load (copies/mL), median [IQR]		177 [110480]		
< 50 copies/mL, (*n*, %)		19 (31.7)		
≥ 50 copies/mL, (*n*, %)		40 (66.7)		
Missing (*n*, %)		1 (1.7)		
Clinical characteristics				
Nodal disease (*n*, %)	34 (45.9)	28 (82.4)	6 (17.6)	0.797
Extranodal disease (*n*, %)	40 (54.1)	32 (80.0)	8 (20.0)	
Cell of origin				
Germinal centre[a] (*n*, %)	39 (52.7)	32 (82.1)	7 (17.9)	0.697
Non-germinal centre[b] (*n*, %)	13 (17.6)	10 (76.9)	3 (23.1)	
Inconclusive (*n*, %)	22 (29.7)	18 (81.8)	4 (18.2)	

Abbreviations: CD, cluster of differentiation; COO, cell of origin; DLBCL, diffuse large B-cell lymphoma; HIV, human immunodeficiency virus; IQR, inter-quartile range; *n*, sample size; SD, standard deviation.
[a]Germinal Centre origin refers to cases expressing CD10 or expressing BCL6 if negative for CD10 and MUM1.
[b]Non-germinal centre origin refers to CD10-negative cases expressing MUM1 and/or BCL6.

3.2 | Biopsy Characteristics

The primary diagnosis of DLBCL was based on 34 (45.9%) nodal and 40 (54.1%) extranodal biopsy sections. Extranodal biopsy sites included: oral cavity/nasopharyngeal ($n=13$, 32.5%), gastrointestinal tract ($n=10$, 25.0%), bone marrow ($n=9$, 22.5%), skin and soft tissue ($n=7$, 17.5%) and breast ($n=2$, 5.0%). On IHC assessment of the cell of origin, the GCB cell of origin predominated ($n=39$, 52.7%). There was no difference in the cell of origin between PLWH and HIV-negative participants ($p=0.697$) (Table 1).

The 'essential' and 'desirable' IHC and molecular results are listed in Table 2. The following 'essential' criteria were commonly requested for diagnosis and sub-classification: CD3, CD10, CD20, Ki-67, BCL-2, BCL-6, MUM-1 and FISH for *MYC* gene rearrangement. The median [IQR] Ki-67 was 90 (85%–95)%. FISH for *MYC* gene rearrangement was performed in 59 (79.7%) participants and was positive in 9 (15.3%). *MYC* gene rearrangement was present in six PLWH and three HIV-negative participants. In all but one participant with *MYC* gene rearrangement, *BCL2* and *BCL6* gene assessment were performed, detecting concomitant *BCL6* gene rearrangement in 1 (11.1%) HIV-negative participant. FISH for *IgH* gene rearrangement was done in three with *MYC* gene rearrangement, and 2 (22.2%) participants had an *IgH* translocation partner (Table 2).

Flow cytometry was performed in 51 (68.9%) participants. The B panel and extended B panel results of the study population are listed in Table 3. Flow cytometry was mostly requested as a staging investigation in bone marrow or the cerebrospinal fluid (CSF) ($n=45$) (60.8%) and as diagnostic investigation in 6 (8.1%). Polymerase chain reaction for IgH gene rearrangement was performed in 11 cases (14.9%), predominantly as part of the staging work-up. Karyotyping was performed on 19 bone marrow samples, including six of the 12 cases with bone marrow involvement. An abnormal complex karyotype was found in three, with common aberrations detected including hyper-diploidy (seen in 2/3), abnormalities of Chromosome 1 (all three cases) and aberrations involving Chromosome 7 (two cases) (Table 3).

TABLE 2 | 'Essential' and 'desirable' criteria for the diagnosis of DLBCL.

Characteristic	Positive (*n*, %)	Negative (*n*, %)
'Essential' criteria		
CD3	0 (0.0)	65/65 (100.0)
CD10	34/54 (63.0)	20/54 (37.0)
CD20	72/74 (97.3)	2/74 (2.7)
Ki-67	73/73 (100.0)	0 (0.0)
BCL-2	47/66 (71.2)	19/66 (28.8)
BCL-6[a]	47/51 (92.2)	4/51 (7.8)
MUM-1	40/67 (59.7)	27/67 (40.3)
c-MYC	0 (0.0)	0 (0.0)
EBER ISH (only in HIV negative)	0 (0.0)	1/1 (100.0)
'Desirable' criteria		
CD79a	7/7 (100.0)	0 (0.0)
CD30	6/13 (46.2)	7/13 (53.8)
CD23	0 (0.0)	1/1 (100.0)
ALK	0 (0.0)	7/7 (100.0)
CD138	0 (0.0)	8/8 (100.0)
FISH[b]		
FISH for *MYC* gene rearrangement[c]	9/59 (15.3)	47/59 (79.7)
FISH for *BCL2* (if the *MYC* gene rearrangement was positive)	0 (0.0)	9/9 (100.0)
FISH for *BCL6* (if the *MYC* gene rearrangement was positive)	1/8 (12.5)	7/8 (87.5)
FISH for *IgH*[d]	2/3 (66.7)	1/3 (33.3)

Abbreviations: ALK, anaplastic lymphoma kinase; CD, cluster of differentiation; DLBCL, diffuse large B-cell lymphoma; EBER-ISH, Epstein–Barr encoding region in situ hybridisation; FISH, fluorescence in situ hybridization; HIV, human immunodeficiency virus; IGH, immunoglobulin heavy locus; MUM1, multiple myeloma oncogene-1; *n*, sample size.
[a]A single case of BCL6 was noted to be equivocal.
[b]FISH was done on 59/74 cases predominantly on tissue sections 57/59 (84.7%) and BMA 2/59 (3.4%).
[c]*MYC* gene rearrangement was unsuccessful in 3/74 cases (4.1%).
[d]FISH for *IgH* gene rearrangement was done in three samples with *MYC* gene rearrangement and was positive in two. *IgH* FISH failed in one patient.

3.3 | Analysis of Test Performed

The 'essential' IHC (as per Cho) was diagnostic for DLBCL in 72 cases (Table S1), with two cases requiring a second-line 'desirable' marker to confirm B-cell lineage. One case was CD79a and HHV8 positive but negative for ALK, CD138 and CD30. CD23 was not done. The other case was positive for CD79a and PAX5, while CD30 and ALK were negative. CD23 and CD138 were not done for this case. FC detected a clonal population in only six cases (8.2%), confirming bone marrow involvement in four during staging procedures and serving as the primary diagnostic modality in two (complemented by IHC on the trephine biopsy). FISH analysis was useful for further diagnostic sub-classification in 9 (15.3%) of the cases it was performed in, while cytogenetics and PCR for IgH gene arrangement complemented the diagnostic in 3 (4.1%) and 4 (5.4%) cases, respectively. Notably, FC failed to detect disease in six of the 12 cases (50.0%) with bone marrow involvement where FC was performed.

TABLE 3 | Flow cytometry and other molecular characteristics of participants with DLBCL.

Characteristic	Positive (*n*, %)	Negative (*n*, %)	
Flow cytometry[a]			
B-Panel			
CD45	6/51 (11.8)	45/51 (88.2)	
CD19	6/51 (11.8)	45/51 (88.2)	23 (31.8)
CD20	5/38 (13.2)	33/38 (86.8)	
CD5	1/43 (2.3)	42/43 (97.7)	
CD10	2/45 (4.4)	43/45 (95.6)	
CD34	0 (0.0)	35/35 (100.0)	
CD38	4/22 (18.2)	18/22 (81.8)	
CD200	1/20 (5.0)	19/20 (95.0)	
Kappa/ Lambda	6/51 (11.8)	45/51 (88.2)	
Extended B-panel			
CD103	0 (0.0)	0 (0.0)	
CD123	0 (0.0)	4/4 (100)	
CD22	4/6 (66.7)	2/6 (33.3)	
CD11c	0 (0.0)	1/1 (100.0)	
CD25	0 (0.0)	1/1 (100.0)	
Cytogenetics			
Chromosome Analysis on staging bone marrow samples[b]	3/19 (15.8)	16/19 (84.2)	
PCR for IgH gene rearrangement[c]			
IgH	4/11 (36.4)	7/11 (63.6)	

Abbreviations: CD, cluster of differentiation; CSF, cerebrospinal fluid; DLBCL, diffuse large B-cell lymphoma; IgH, immunoglobulin heavy locus; *n*, sample size; PCR, polymerase chain reaction.

[a]Flow Cytometry was done on 51/74 patients, of which samples analysed include the following: bone marrow aspirate 39/51 (76.5%), cerebrospinal fluid 6/51(11.8%), lymph node fine needle aspiration 2/51 (3.9%), pleural fluid 2/51 (3.9%), peripheral blood 2/51 (3.9%) and 1 collected from an unspecified site 1/51 (1.9%). One patient had flow performed on both a BMA and CSF, and another on both a BMA and a pleural fluid sample.

[b]Chromosome analysis failed for one case. For the three positive results, the following aberrations were noted: Study Number 26; 46,X,-Y,add (1) (p?31), del (1) (q?21), der (7) add (7) (p?22) add (7) (q?36), inv. (9) (p12q?q1). Study Number 40; 61–69 < 3n , XXX, +X, +X, +X, +X, +X, dic(1;17) (p?13;p?13), dup (7) (q?22q?11.1),-8,-10,-13. Study Number 41; 50–53, XY, +X, +der (1) del (1) (p?31) inv. (1) (p?31q?12), +5, der(8)t(8;8) (p?21;q?23), i (17).

[c]Polymerase Chain Reaction (PCR) was done on 11/74 cases. The sample types included bone marrow 5/11 (45.5%), CSF 4/11 (36.4%), fine needle aspiration 1/11 9.1% and pleural fluid 1/11 (9.1%).

3.4 | Analysis of the Reasons for Test Omission

As regards the 'essential' IHC, testing for c-MYC was not performed in 74 (100%) because of the non-availability of the testing at the NHLS over the study period. Additionally, EBER ISH was not done in 13 (92.9%) of the HIV-negative cases, possibly due to a lack of pathologist awareness (Table 4). Furthermore, CD10 and BCL6 were not done in 12 and 14 patients, respectively, owing to a prolonged reagent stock-out over ~4 months during the study period. FISH for *MYC* rearrangement was unsuccessful in 3 (4.1%).

3.5 | Cost Assessment

At the NHLS, the cost for the panel recommended by Cho [17] (including IHC and FISH) is $750. The cost of the average work-up (IHC and FISH) performed in this study population was $383 (Table 5). The average cost of additional tests performed, including FC, cytogenetics and PCR for IgH rearrangement, was $177.

4 | Discussion

HIV infection has been linked to the occurrence of large B cell lymphoma in South Africa, where DLBCL is the most prevalent subtype of NHL in adults [3]. In this current study, 60 (81.1%) of the cases were diagnosed in PLWH, in keeping with the national HIV statistics [6]. DLBCL is usually a late event during HIV infection, and this study group was characterised by a low median CD4 count of 143 cells/μL and high HIV viral load in 40 (66.7%), thus affirming published data. DLBCL was most common amongst black males, with a mean age of 44 years. This is in line with a previous study from Southern Africa, which found 57.5% of patients with NHL to be male, with a median age of 41 years [4]. Likewise, in a study from Nigeria, of 152 participants with NHL, the male to female ratio was 2:1, with a mean age of 44 years [18]. These authors also highlighted that DLBCL was the commonest subtype affecting PLWH. Future studies to better understand the sex distribution in NHL, specifically DLBCL, would be of interest.

Most of the cases of DLBCL in this study were of extra-nodal ($n = 40$, 54.1%) origin; of which the oral cavity/nasopharynx, bone marrow, and gastrointestinal were the commonest sites. This finding is somewhat higher than results from the developed world, where extra-nodal disease accounts for 24%–48% of all NHL [19]. Similarly, in a study at a tertiary hospital in Cape Town, South Africa, up to 40% of DLBCL were of extra-nodal origin, with the gastrointestinal tract being the most commonly affected site [20]. Extra-nodal site involvement is a poor prognostic factor, particularly if more than one extra-nodal site is involved [7].

The WHO and ICC Classifications categorise DLBCL with respect to prognostic significance according to the cell-of-origin into non-germinal centre and GCB subtypes [1, 11, 12]. While gene expression profiling provides a more accurate assessment of the cell-of-origin, this technique is costly and not readily available for routine testing. Immunohistochemistry is currently the most widely used method in routine practice owing to its

TABLE 4 | Reasons tests are not being performed.

Reasons	Total test not done/resulted ($n=74$, %)	Insufficient/no sample (n, %)	Reagent out of stock/not available (n, %)	Test failure (n, %)	No reason evident (n, %)
Immunohistochemistry					
'Essential'					
CD3	9 (12.2)				9 (100)
CD10	18 (24.3)	1 (5.6%)	12 (66.7%)		4 (2.2)
Ki67	1 (1.4)				1 (100)
BCL2	8 (10.8)	1 (12.5)			7 (87.5)
BCL6	23 (31.1)	1 (4.4%)	14 (60.9)		8 (34.8)
MUM1	7 (9.5)	1 (14.3)			6 (85.7)
c-MYC	74 (100.0)		74 (100.0)		
EBER (in HIV negative)	13/14 (92.9)				13 (HIV negative)
'Desirable'					
CD79a	67 (90.5)				67 (90.5)
CD30	61 (82.4)				61 (82.4)
CD23	73 (98.6)				73 (98.6)
ALK	67 (90.5)				67 (90.5)
CD138	66 (89.2)				66 (89.2)
FISH					
'Essential'					
MYC rearrangement	18 (24.3)			3 (16.7)	15 (83.3)
Flow cytometry					
B and/or extended B panel	23 (31.1)				23 (100)
Cytogenetics	55 (74.3)			1 (1.8)	54 (98.2)
PCR for IgH gene rearrangement	63 (85.1)				63 (100)

Abbreviations: DLBCL, diffuse large B-cell lymphoma; FISH, fluorescence in situ hybridization; IgH, immunoglobulin heavy locus; n, sample size; PCR, polymerase chain reaction.

accessibility and cost-effectiveness. IHC for CD10, BCL6 and MUM1 according to the Hans algorithm has shown an 80% correlation with gene expression profiling [21]. On analysis of the cell-of-origin by IHC, the GCB cell of origin predominated in this study population ($n=39$, 52.7%). This is consistent with a previous report which showed a predominance of GCB cell of origin in DLBCL in the setting of HIV on gene expression profiling analysis [22]. The authors also reported co-expression of CD10, BCL6 and MUM1 in 19% for PLWH and 6% for HIV-negative participants [22] Nonetheless, the cell-of-origin has not been associated with prognostic significance in DLBCL in the setting of HIV [23–25]. Additional IHC for EBER-ISH, BCL2 and c-MYC have also demonstrated prognostic value in DLBCL in the setting of HIV [26, 27]. BCL2 was performed in the majority of cases (89.2%); however, the 'essential' IHC, c-MYC as well as EBV in the HIV-negative cases were not performed as these IHC reagents were not available at the institution. In a resource-limited setting, IHC for EBV, BCL2 and c-MYC may be of greater prognostic value than IHC for the cell-of-origin. Large studies are warranted to determine the prognostic value of these IHC tests to further refine 'essential' IHC testing.

IHC alone, however, does not show the genetic diversity of DLBCL, and molecularly defined subtypes of DLBCL with distinct molecular and clinical features have been introduced, namely DLBCL/high-grade B-cell lymphoma with *MYC* and *BCL2* rearrangement (WHO 5th edition) [1]. The selection of molecular tests for DLBCL must be made with careful consideration to avoid placing unnecessary economic strain. A mean of 1 ± 1 'essential' FISH test was performed. *MYC* rearrangement

TABLE 5 | Cost assessment.

Diagnostic tests	Cost per unit ($)	Mean ± SD	Total cost ($)
Tests performed at NHLS			
Immunohistochemistry markers	30		
'Essential'		6.1 ± 1.1	183
'Desirable'		0.5 ± 0.8	15
Other markers		1.4 ± 2.0	42
Total (IHC)		8.0 ± 2.6	240
FISH analysis	110		
Total (FISH)		1.3 ± 1.1	143
Total (IHC and FISH)			383
The recommended panel by Cho (2022) and the 4th edition of WHO [11, 17]			
IHC markers	30		
'Essential'		9	270
'Desirable'		5	150
Total (IHC)		14	420
FISH analysis	110		
'Essential'		1	110
'Desirable'		2	220
Total (FISH)		3	330
Total (IHC and FISH)			750
The recommended panel by Cho (2022) and the 5th edition of WHO [1, 17]			
IHC markers	30		
'Essential'		9	270
'Desirable'		6	180
Total (IHC)		15	450
FISH analysis	110		
'Essential'		1	110
'Desirable'		3	330
Total (FISH)		4	440
Total (IHC and FISH)			890
Other investigations			
Flow cytometry	13		
Number of markers		8.7 ± 8.3	113
Cytogenetics	192	0.26 ± 0.4	50
PCR analysis—IgH	93	0.15 ± 0.36	14
Total (FC, CYTO and PCR)			177

Abbreviations: FISH, fluorescence in situ hybridization; IgH, immunoglobulin heavy locus, NHLS, National health laboratory services, PCR, polymerase chain reaction; SD, standard deviation; WHO, World Health Organization.

was detected in 9 (12.2%) cases in this cohort. The detection rate of *MYC* rearrangements was similar to reports from high-income countries, which have reported rates of 8%–14% of DLBCL [1, 11]. Subsequent FISH testing was performed for dual *IgH-MYC* fusion, *BCL2* and *BCL6* translocations. There were no cases of *MYC* and *BCL2* rearrangement and only one case of

MYC/BCL6 co-rearrangement. *IgH-MYC* fusion was positive in 2 (22.2%) of the nine cases (66.7% of those tested), a finding that has been associated with a poor prognosis and has subsequently been recommended as a desirable parameter by the 5th edition of the WHO Classification [1, 11]. Other non-Ig partners involving *MYC*, such as *BCL6* and *PAX5*, comprise < 5% [1].

A mean of 6.1 ± 1.1 'essential' IHC markers were performed, whereas 'desirable' IHC markers were rarely requested. For 'essential' IHC, c-MYC was not performed due to the unavailability of IHC reagents. The reason for the failure to perform EBV analysis in most of the HIV-negative participants may be attributed to a lack of pathologist awareness. This testing was not indicated in the PLWH, as patients with underlying immunodeficiency were excluded from the entity of EBV-positive DLBCL in the WHO 4th edition. Assessment for underlying oncogenic viral infection (EBV and KSHV) in lymphomas arising in immune deficiency/dysregulation has now been added to the WHO 5th edition as additional 'desirable' testing [1]. The 'essential' IHC stains were diagnostic for DLBCL in 72 (97.3%) cases in the presence of medium- to large-sized cells with a diffuse pattern. CD20 expression was absent in 2 (2.7%) cases, necessitating further IHC to prove B-cell lineage. Cell-of-origin assignment was possible in 39 (70.3%) cases, with definitive classification hampered in some of the cases by reagent stock-outs. This further highlights the challenges of comprehensive work-up in a resource-constrained environment. Lastly, FC, PCR for IgH and cytogenetics were diagnostic in 8.2% of cases.

The average number of IHC markers performed in this cohort was less than that advised in the panel recommended by Cho owing to EBER, c-MYC and most of the 'desirable' markers not being done [17]. Nonetheless, c-MYC expression with or without BCL2 co-expression has not consistently demonstrated prognostic value in DLBCL in the setting of HIV, and the value added by this marker is therefore tenuous [26, 27]. A complete panel including both the 'essential' and 'desirable' IHC would cost approximately 50% more per patient ($750) as compared to the IHC and FISH costs incurred in this cohort. Since a diagnosis was successfully made in all cases in this study, the need for further testing is called into question, particularly in the resource-constrained setting. Notably, the 5th edition of the WHO recommends IHC for EBV and KSHV in immunodeficiency-associated DLBCL in addition to the current testing. This will incur an additional cost of $60 per patient. The feasibility and value of doing these markers in resource-limited centres requires thoughtful consideration. A pragmatic approach would be to undertake the 'desirable' testing only when 'essential' markers are inconclusive, as seems to have been done in real-world practice in this cohort. Figure S1 shows the relative cost of this strategy for the 4th and more recent 5th edition of the WHO. Furthermore, CD21 to rule out follicular large B-cell lymphoma and cyclin D1 to exclude pleomorphic mantle cell lymphoma should also be considered to exclude differential diagnoses of DLBCL.

Additional tests such as FC and PCR were of limited diagnostic value yet relatively costly in this cohort. However, this largely reflects the cost of disease staging, as opposed to diagnosis. Education of pathologists, as well as establishment of criteria for submitting these ancillary investigations, is recommended to ensure uniformity and to limit unnecessary costs.

The results of this study must be interpreted considering the following limitations. First, the study participants were not classified according to the 5th edition of the WHO because testing for EBV and KSHV was not routinely performed in PLWH in 2019–2022. During this period, participants were also diagnosed according to the 4th edition of the WHO. Second, in a small proportion, no reason was recorded in the laboratory records for not performing the recommended 'essential' diagnostic tests.

Limited availability of IHC and molecular diagnostic tests, as well as expertise in low-and middle-income countries, hampers the diagnosis and classification of DLBCL. The advocated 'essential' panel of IHC stains and molecular investigations (FISH for *MYC* gene rearrangement) in addition to 'desirable' tests, where necessary, enabled the accurate and cost-effective diagnosis of DLBCL in this cohort and is recommended for other parts of the world with limited resources. Additional tests such as FC, PCR for IgH and cytogenetics should be carefully considered in the staging work-up of DLBCL. Future research focusing on an integrated cost assessment that includes clinical, histopathological and molecular profiling is warranted to enhance the selection of diagnostic tests, particularly in low-resource settings. This approach would help optimise healthcare resource allocation while ensuring an accurate diagnosis.

Author Contributions

G.A.M. contributed to study design, data collection, entry and analysis and writing of the manuscript. E.S. and J.V. contributed to study design and critical review of the manuscript. All authors revised the manuscript and approved the final version.

Ethics Statement

This study was approved by the Human Research Ethics committee of the University of the Witwatersrand (Protocol number: M240438).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.