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Research Article

BCL6 Protein Expression Identifies Cases without *BCL6* Gene Rearrangement in Non-Germinal Centre Diffuse Large B- Cell Lymphoma Cases

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Abstract

BCL6 gene *r*earrangements are the most frequent cytogenetic abnormality in Diffuse Large B-Cell Lymphoma (DLBCL), occurring in up to 64% of cases, suggesting a poorer prognosis and response to therapy. The aim of this study was to calculate the accuracy and predictive power of BCL6 IHC in identifying *BCL6* gene rearrangements. A search for DLBCL cases was performed on the laboratory information system. We retrospectively analysed 46 cases of DLBCL, and correlated BCL6 protein expression with gene rearrangement status. Of the 46 cases, 39 (84.78%) showed positive BCL6 protein expression by IHC. In comparison, only 10 (21.74 %) samples presented with gene rearrangements. There were 8 cases positive for BCL6 IHC with gene translocations and 5 cases were negative for both protein expression and gene rearrangements. BCL6 IHC had a sensitivity of 80% and a specificity of 14%. Furthermore, the Positive Predictive Value (PPV) and Negative Predictive Value (NPV) were 21% and 71%, respectively. *BCL6* rearrangement was more common in cases with a Non-Germinal Centre (GC) Cell of Origin (COO) (45.5%) as compared to the GC group (14.3%). When stratifying the analysis according to the COO, the sensitivity and NPV of BCL6 IHC in the non-GC group were both 100%, while the same parameters were considerably poorer in the GC cases. BCL6 protein expression did not correlate with the presence of *BCL6* gene rearrangements in HIV related DLBCL cases. However, lack of BCL6 protein expression may be used to identify cases without a *BCL6* gene rearrangement, particularly in non-GC COO.

Keywords: NPV; PPV; Sensitivity; Specificity; Immunohistochemistry; Fluorescence in-situ hybridization

Introduction

Rearrangements of the *BCL6* gene are the most frequent cytogenetic abnormality in Diffuse Large B-Cell Lymphoma (DLBCL), occurring in up to 64% of HIV unrelated cases [1-4] and 20% of HIV related cases [5]. The *BCL6* gene is found on chromosome 3q27, and encodes a 96 kDa protein with C-terminal zinc-finger motifs and an N-terminal protein-protein interacting domain [1,6]. The *BCL6* chromosomal translocation partners are many; a phenomenon termed promiscuous translocation, and may involve a variety of Immunoglobulin (IG) genes on differing loci [7].

BCL6 protein expression preferentially occurs in germinal centre B cells [8] and is thus included in the Hans algorithm, classifying DLBCL into Germinal Centre (GC) and non-GC phenotypes [9]. Protein expression of BCL6 is detected in up to 79% of HIV unrelated DLBCL cases [4,10] and 56% of HIV related DLBCL [11], with a strong nuclear staining pattern [12].

Gene rearrangements are detected using Fluorescence In-Situ Hybridization (FISH), which is technically intensive, time-consuming and expensive [13]. In contrast, Immunohistochemistry (IHC) is relatively cheaper, widely available and easier to interpret [13].

Diagnostic tests are used to establish the absence or presence of a condition. In contrast, screening tests are used to determine the risk of acquiring the condition. Screening tests are usually more accessible, less invasive and less expensive compared to diagnostic tests [14]. IHC has been previously shown to be a good screening tool that can detect some gene rearrangements with high accuracy as in the case of *MYC* [15-17]. The

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accuracy of a screening tool relative to a known standard test is informed by calculating sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) [14].

Previous studies on DLBCL found no association between *BCL6* rearrangements and BCL 6 protein expression [11,12,18]. However, the values for accuracy and predictive power of BCL6 IHC were not calculated. The aim of this study was to determine the accuracy and predictive power of BCL6 IHC in identifying *BCL6* gene rearrangements in DLBCL cases from South Africa.

Materials and Methods

Ethics Committee Approval and Patient Consent

This study was approved by the University of the Witwatersrand Human Research Ethics Committee (R14/49), according to the principles of the Declaration of Helsinki.

Cases Selection

A total of 46 cases of Diffuse large B-Cell Lymphoma (DLBCL) were retrieved from the TrakCare laboratory database of the Department of Anatomical Pathology for 2017. Cases with both FISH and IHC data on BCL6 were included in the study. The demographic data, IHC staining profile and FISH results were extracted from pathology reports and analysed.

Immunohistochemical Analysis and Scoring

IHC was performed as part of the diagnostic work-up. Briefly, DLBCL cases were sectioned at 4µm and baked at 56°C for at least an hour. The slides were pre-treated with heat induced epitope retrieval with high pH retrieval solution, using the Automated Link 48 (Dako, Glostrup, Denmark) instrument. Slides were blocked with hydrogen peroxide for 10 min prior to the application of primary antibody. The monoclonal antibody BCL6 (PG-B6P, Dako, Glostrup, Denmark) was used as part of the Hans Criteria [9]. The Hans algorithm classifies DLBCL cases into GC and non-GC subtypes, using the immunohistochemical staining patterns of CD10, BCL6 and MUM1. Visual detection was achieved using the Dako Envision horse radish peroxidase for 20 min.

IHC results were scored according to the Hans classification by using a 30% cut-off value [9]. Slides with ≥30% tumour staining were considered positive, while those with <30% tumour staining or with weak or focal staining were scored negative.

FISH Analysis and Scoring

BCL6 FISH analyses were requested during the diagnostic workup, if required. FISH analysis was performed on unstained 2µm tissue sections using a Vysis LSI BCL6 dual colour break-apart probe (Abbott Molecular Inc., Desplaines, IL, USA). Tumour areas were defined by haematoxylin & eosin stained tissue sections from representative formalin-fixed, paraffin-embedded tissue blocks. Images were captured with Cytovision 4.0 (Leica Biosystems Inc., Buffalo Grove, IL, USA) on an Olympus BX61 fluorescence microscope (Olympic Scientific Solutions, Waltham, MA, USA) as previously described [19].

Data Analysis

Sensitivity, specificity Positive Predictive Value (PPV) and Negative Predictive Value (NPV) were calculated based on [20], as follows:

Sensitivity= true pos/(true pos+ false neg) x 100

Specificity= true neg/ (true neg+ false pos) x100

PPV= true pos/ (true pos + false pos) x100

NPV= true neg/ (true neg+ false neg) x100

"True positive" was defined as cases with gene rearrangements and positive IHC expression

"True negative" was defined as cases with no gene rearrangements and negative IHC expression.

"False positive" was defined as cases with no gene rearrangements and positive IHC expression

"False negative" was defined as cases with gene rearrangements and negative IHC expression

Results

Our cohort consisted of 46 DLBCL cases diagnosed in 2017 (Table 1). The average age of the cohort was 40±8.996 years with 30.43 % (n=14) female and 69.57 % (n=32) male population, respectively. Three cases (6.52%) were diagnosed as double hit lymphomas with both *MYC* and *BCL6* translocations. *MYC* was translocated in 10 (22.73 %) of the DLBCL cases. There were 35 (76.09%) cases with GC subtype and 11 (23.91%) with non-GC subtype phenotypes. Only 42 cases (91.30%) were tested for HIV infection, which was seropositive in 36 (85.71%) of the cases. The median CD4 count was 183±247cells/uL with more than half of the cases with CD4 counts less than 200cell/µL. The median viral load was 706.5±273 880 copies/mL, with majority of the cases with less than 1000 copies/mL (Table 1).

Table 1: A summary of the demographics of the study cohort.

Characteristics	
Age (Mean ± SD)	40± 8.996296
Sex n=46	
Male	32(69.57 %)
Female	14(30.43 %)
Diagnosis n=46	
DLBCL	43(93.48 %)
DLBCL (DHL)	3(6.52 %)
Variant n=46	
GC	35(76.09 %)
Non-GC	11(23.91 %)
HIV n=42	
Neg	6(14.29 %)
Pos	36(85.71 %)
Median CD4 (cells/uL) +/- IQR n=33	183±247
< 200 cell/ul	19(57.55%)
>200 cell/ul	14(42.42%)
Median viral load +/- IQR (copies/ml) n=30	706.5±273880
LDL (≤20 copies/ml)	4(13.33%)
<1000 copies/ml	12(40.00%)
1000-100 000 copies/ml	6(20.00%)
>100 000 copies/ml	8(26.67%)
BCL6 IHC staining n=46	
Neg (including focal or weak staining)	7(15.22%)
Pos	39(84.78%)
BCL6 translocated n=46	
Neg	36(78.26 %)
Pos	10(21.74 %)
MYC translocated n=44	
Neg	34(77.27 %)
Pos	10(22.73 %)

DLBCL: Diffuse Large B Cell Lymphoma, DHL: Double Hit Lymphoma, neg: Negative, pos: Positive

Table 2: Statistical analysis of how BCL6 IHC can predict the presence of BCL6 translocation in DLBCL cases.

True positive (IHC+, FISH+) 8	False positive (IHC+, FISH-) 31
False negative (IHC-, FISH+) 2	True negative (IHC-, FISH-) 5
Sensitivity = true pos	(true pos + false neg) x 100 = 80%
Specificity = true neg	(true neg + false pos) x 100 = 14%
PPV = true pos	(true pos + false pos) x 100 = 21%
NPV = true neg	(true neg + false neg) x 100 = 71%

Table 3: Statistical analysis of how BCL6 IHC can predict the presence of BCL6 translocation in DLBCL subtypes.

	non-GC	GC
True Pos	5	3
False Pos	3	28
True neg	3	2
False Neg	0	2
Sensitivity	100%	60%
Specificity	50%	7%
PPV	63%	10%
NPV	100%	50%

Of the 46 samples, 39 (84.78%) showed positive BCL6 protein expression by immunohistochemistry. In comparison, only 10 (21.74 %) contained *BCL6* translocations as evidenced by FISH (Table 1).

There were 8 case which showed both BCL6 protein expression and BCL6 rearrangements (Table 2). There were 5 cases which were negative for both BCL6 expression and gene rearrangements. There was a single case with negative protein expression which had BCL6 gene rearrangements. There were nine false positive cases which were positive for BCL6 protein expression but negative for gene rearrangements.

The ability of BCL6 IHC to correctly identify BCL6 gene rearrangement cases (sensitivity) was 80%. The ability of IHC to correctly identify cases without a gene translocation (specificity) was 14%. The likelihood that a case had a gene translocation given a positive IHC result (PPV) was 21%. The likelihood that a case did not have a gene translocation given a negative IHC result was (NPV) 71% (Table 2).

We also determined whether DLBCL subtype affects the calculated predictive values of BCL6 IHC. In the GC group of cases (n=35), there were 5 cases (14.28%) with BCL6 translocations and 31 cases (88.6%) with BCL6 IHC positivity. In contrast, the non-GC group (n=11), had 5 cases with BCL6 translocations (45.45%) and 8 (72.73%) with BCL6 IHC positivity.

The sensitivity and NPV of BCL6 IHC in the non-GC group were both 100% and correlation was overall superior in this subgroup, while the same correlation of these parameters were lowered in the GC group (Table 3).

Discussion

The study cohort had a mean age of 40 years which is consistent with the demographics of the HIV related DLBCL population [21]. The male predominance of this cohort is in contrast to the usual female predominance, which may be due to sampling procedures [22]. The median CD4 count of the study cohort was approximately 183cells/uL - similar to the average observed by the Pather study [23]. The median viral load was within the range seen in the South African population [24,25].

BCL6 is expressed in the nuclei of mature B cells in germinal centres as well as in their transformed counterparts in DLCL biopsies [26]. *BCL6* is essential for GC formation and promotes the development of B-cell lymphomas [27]. *BCL6* overexpression is caused by gene translocations and somatic mutations in the 5' non-coding region. Gene translocation causes the promoter of the *BCL6* gene to be substituted, thus leading to deregulated expression [28].

The *BCL6* gene is one the most frequently translocated genes in DLBCL with prevalence rates of up to 64% [4,29]. This study observed a 21.7% prevalence rate in DLBCL cases, which is similar to prevalence from cohorts with high HIV seropositivity rates [11,29].

The prognostic effects of *BCL6* translocations are contentious, with studies either showing no effect [4,30] or a worse prognosis [31]. A meta-analysis of 22 studies involving a cohort of 3037 patients by Li et al [32] conclusively showed that *BCL6* gene rearrangements indicate a poorer prognosis, but only in patients treated with rituximab containing regimens. The unfavourable prognosis conveyed by *BCL6* overexpression may be executed by increasing resistance to chemotherapeutic through an increase in the antioxidant defence systems [8].

BCL6 protein expression, both at the mRNA and protein level, is one of the predictors of outcome in DLBCL cases [33-35]. Positive expression predicts longer overall survival times compared to negative expression [4,34]. Iqbal (2007) observed that patients with an overexpression of BCL6 at both mRNA or protein levels; had a significantly better overall survival, irrespective of IHC cut-offs used or DLBCL subtype (GC or ABC). Our study has shown BCL6 protein expression in 84% of cases, which is slightly higher than the range previously described (detected in 45-79% of DLBCL cases [10,11,36].

As reported previously [27], we found the prevalence of BCL6 translocations to differ depending on the DLBCL subtype, with lower prevalence rates in the GC subgroup (14.28%) as compared to the non-GC group (45.45%). However, the BCL6 protein positivity rate did not differ by subtype, suggesting that there may be other ways that BCL6 protein is overproduced.

Clinical tests are used to confirm or refute the presence of a condition. Ideally such tests correctly identify all patients with the condition, and similarly correctly identify all patients who are condition free. When evaluating a clinical test, the terms sensitivity and specificity are used, while the terms PPV and NPV are used when considering the value of a test to a clinician [20,37,38].

Sensitivity and specificity indicate the concordance of a test with respect to a chosen standard reference. In our study cohort, BCL6 IHC expression did not correlate with *BCL6* gene rearrangement, as previously presented [11,39]. PPV and NPV, respectively, indicate the likelihood that a test can successfully identify people with or without the condition [14]. In our study, NPV was very high (100%) in the non-GC group. This suggests that FISH for *BCL6*-gene rearrangement is not indicated in patients with a non-GC immunophenotype with negative BCL6 IHC in the South African setting. However, this result is based on a small sample size (11 patients), and requires validation in a larger cohort.

Conclusion

This study aimed to determine the accuracy and predictive power of BCL6 IHC in identifying *BCL6* gene rearrangements in DLBCL cases. We found that BCL6 protein expression did not

correlate with the presence of *BCL6* gene rearrangements in HIV related DLBCL. However, failure to express BCL6 protein may be used to identify cases without a *BCL6* gene rearrangement, particularly in DLBCL with a non-GC immunophenotype.

Statements and Declarations

Ethics Approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Human Research Ethics Committee of (R14/49).

Conflicts of Interest

The authors report there are no competing interests to declare.

Author Contributions

All the authors contributed equally to the conception, data acquisition, analysis and drafting the work and have final approval of the version to be published.

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The study did not require funding.

Data Availability Statement

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

Disclaimer

A statement that the views expressed in the submitted article are his or her own and not an official position of the institution or funder.

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