ORIGINAL ARTICLE

Plasmablastic lymphoma versus diffuse large B cell lymphoma with plasmablastic differentiation: proposal for a novel diagnostic scoring system

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Abstract High-grade non-Hodgkin's lymphomas with plasmablastic differentiation are frequently encountered in HIV-positive patients. Differentiating between diffuse large B cell lymphoma (DLBCL) with plasmablastic differentiation and true cases of plasmablastic lymphoma (PBL) is sometimes challenging, particularly as a substantial overlap in immunphenotype exists between late-stage B cell neoplasms and PBL. This study sought to develop an immunohistochemical panel to more reliably distinguishing between PBL and DLBCL with plasmablastic differentiation. Thirty-nine CD20-negative, ALK-negative, HHV8-negative non-Hodgkin's lymphomas with plasmablastic differentiation defined by their morphological features, high proliferation index and positivity for MUM1/IRF4 and PRDM/Blimp1 protein expression were compared regarding their protein expression profiles, viral status and c-MYC-gene aberrations. These lymphomas were subsequently divided in two groups utilising CD10 and Pax5. Tumours without reactivity for either of these markers exhibited higher expression of CD138 and CD117 frequently used as the plasma cell (PC) markers, whilst tumours with reactivity for one or both markers showed a significantly higher expression of CD38 and MYC-gene aberrations. A novel diagnostic scoring system which includes the immunohistochemical expression of CD10 and Pax5 is proposed to differentiate between DLBCL with plasmablastic differentiation and true cases of PBL.

Keywords Plasmablastic lymphoma · Diffuse large B cell lymphoma · Plasmablastic differentiation · Diagnostic scoring · HIV

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Introduction

B cell non-Hodgkin's lymphomas (NHLs) associated with HIV-infection share certain clinical and pathological features. The tendency to exhibit plasmablastic differentiation is perhaps one of the most peculiar microscopic phenomena exhibited by these neoplasms. The entity of plasmablastic lymphoma (PBL) was described as a highly aggressive neoplasm affecting the oral cavity of HIV-infected individuals [9, 16, 21, 22]. Although previously classified as a sub-type of diffuse large B cell lymphoma (DLBCL), PBL is currently recognised as a distinct entity and acknowledged to infrequently also occur outside the oral cavity and in the absence of HIV infection [44]. Transition of a B lymphocyte through the plasmablastic differentiation stage to become a mature plasma cell (PC) is a complex process characterised by loss of B cell antigens and acquisition of PC differentiation markers. PBL is considered the prototype of large B cell NHL with plasmablastic differentiation present throughout the neoplastic proliferation. The distinction of DLBCL with significant

plasmablastic differentiation from cases of true PBL, however, remains problematic in many cases. The relative lack of specific diagnostic criteria has contributed to a significant overlap and sometimes even confusion between these lymphoma entities in the literature. As patients diagnosed with PBL appear to have a significantly poorer overall survival than patients with DLBCL [40], reproducible distinction between these tumours is important from a prognostic point of view.

Owing to the high prevalence of HIV-infection, high-grade lymphomas with plasmablastic differentiation are encountered frequently amongst patients in South Africa [3, 4]. It was therefore decided to perform a 5-year retrospective analysis of oral cavity NHL diagnosed as PBL. The aim was to develop a diagnostic algorithm to more accurately distinguish true PBL from CD20-negative DLBCL with plasmablastic differentiation.

Materials and methods

Inclusion criteria

All CD20-negative, ALK-negative, HHV8-negative NHLs demonstrating typical morphological features of plasmablastic differentiation as defined by the WHO [44], immunoreactivity for MUM1/IRF4 and PRDM1/Blimp1 protein and with a high proliferation index on Ki67 staining were retrieved from the archives of the Department of Oral Pathology and Oral Biology, University of Pretoria, South Africa. Permission for this work was granted by the main Ethics Committee of the Faculty of Health Science (96/2011).

Immunohistochemistry

All retrieved cases were subjected to a battery of immunohistochemical markers (Table 1), which included additional B cell markers such as CD10, CD19, CD79a, bcl6, and Pax5. Three micron sections from formalin-fixed paraffin wax embedded (FFPE) tissue blocks were dewaxed and hydrated to distilled water. Heat-induced epitope retrieval using a Pascal pressure chamber (Dako Cytomation, USA) was performed on the sections buffered at pH 6 (citric acid) or pH 9 (EDTA or TRIS/EDTA) depending on the specific antigen. Sections were treated with hydrogen peroxide to quench endogenous peroxidase activity and then incubated with their respective anti-sera. The antibodies were detected with either Envision+ System-HRP Labelled Polymer (Dako, Glostrup, Denmark) or Novolink Polymer Detection Kit (Novocastra, Leica Biosystems, UK) according to the manufacturer's recommendations and visualised with diaminobenzidine (DAB) chromogen (Dako, Glostrup, Denmark). Sections were counterstained with haematoxylin, and simultaneous positive and negative controls were performed in all cases. Brown granular

staining of cell membranes (CD3, CD10, CD19, CD20, CD38, CD45, CD56, CD79a, CD138 and CD117), cytoplasm (ALK) or nuclei (Pax5, Ki67, bcl6, MUM, ALK, Blimp and HHV8) was regarded as positive when 20 % or more of tumour cells showed signalling. Intensity of positive staining was noted as weak or strong. All analyses were undertaken in non-necrotic tumour areas by two independent observers.

In situ hybridisation

In situ hybridisation was performed on 3-μm FFPE tissue block sections using EBV-encoded small nuclear RNA (EBER1/2) probes (Novocastra, Leica Biosystem, UK) for EBV, nuclear RNA probes (Novocastra, Leica Biosystem, UK) for HHV8 and INFORM® cytoplasmic lambda mRNA and kappa probe for kappa and lambda (Ventana Medical Systems, USA). The sections were dewaxed and hydrated to distilled water and digested with Proteinase K solution (Dako, Glostrup, Denmark) for 3-6 min at room temperature. Twenty microlitres of appropriate fluorescein-labelled EBV, HHV8, kappa and lambda probe were added to the different slides, cover slipped, denatured at 65 °C for 15 min and hybridised at 37 °C for 2 h. The hybridised EBV and HHV8 probes were detected using a universal ISH detection kit (Novocastra, Leica Biosystems, UK) according to the manufacturer's guidelines. The detection of kappa and lambda probes was performed using the ISH iVIEW Blue Detection kit (Ventana Medical Systems, USA) according to the manufacturer's guidelines. Sections were counterstained with haematoxylin and mounted with Faramount Aqueous Mounting Media (Dako, Glostrup, Denmark). Appropriate positive and negative controls were performed in all cases. Nuclear staining was regarded positive for EBV and HHV8, whilst cytoplasmic and/or membranous staining was regarded as positive for kappa/lambda light chains. Light chain restriction was defined as positivity for either kappa or lambda but no reactivity for the other.

Fluorescent in situ hybridisation

Cases were screened for breaks in the *MYC* gene using Vysis LSI dual-colour break apart (BA) rearrangement probes (Abbott Molecular Inc, IL, USA) and for MYC/IGH translocations using Vysis LSI IgH/Myc:CEP 8 tri-colour translocation probes. Four-micron sections were deparaffinised, rinsed in absolute alcohol and air-dried. After pre-treatment and digestion, 10 μL of probe mixture (7 μL buffer solution, 2 μL distilled water, 1 μL probe) were applied to the slides. Denaturation and overnight hybridisation was performed in a thermocycler (Thermobrite, Abbot Molecular Illinois, USA) followed by a post hybridisation wash and counterstaining in 200 ng/mL 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI) (Merck, Darmstadt, Germany) on the sections, cover

Table 1 Summary of antibodies and in situ hybridisation probes utilised in this study

	Clone	Supplier	Dilution	HIER	Detection system
Antibody (IHC)					
CD20cy	L26	DAKO Glostrup Denmark	RTU	Citric pH6	Dako
ALK	ALK1	DAKO Glostrup Denmark	RTU	TRIS/EDTA pH9	Dako
CD45	2B11+PD/26	DAKO Glostrup Denmark	RTU	Citric pH6	Dako
CD3	PS1	Novocastra Leica Biosystems, UK	RTU	EDTA pH9	Dako
MUM1	MUM1p	DAKO Glostrup Denmark	RTU	TRIS/EDTA pH9	Dako
Blimp-1	3H2-E8	Novus Biologicals, Littleton, CO USA	1:200	TRIS/EDTA pH9	Novocastra
Ki67	MM1	Novocastra Leica Biosystems, UK	RTU	TRIS/EDTA pH9	Dako
BCl6	PG-B6p	DAKO Glostrup Denmark	RTU	TRIS/EDTA pH9	Novocastra
CD10	56C6	DAKO Glostrup Denmark	RTU	TRIS/EDTA pH9	Dako
CD19	LE-CD19	DAKO, Glostrup Denmark	RTU	Tris/EDTA pH9	Novocastra
CD79a	JCB117	DAKO Glostrup Denmark	RTU	Citric pH6	Dako
Pax5	DAK-Pax5	DAKO Glostrup Denmark	RTU	Tris/EDTA pH9	Novocastra
CD38	SPC32	Novocastra Leica Biosystems UK	1:100	Citric pH6	Dako
CD138	MI15	DAKO Glostrup Denmark	1:50	Citric pH6	Dako
CD56	123C3	DAKO Glostrup Denmark	RTU	TRIS/EDTA pH9	Dako
CD117	polyclonal	DAKO Glostrup Denmark	1:400	EDTA pH9	Novocastra
Probe (ISH)					
HHV8	mRNA	Novocastra Leica Biosystems, UK	RTU	Proteinase K	Novocastra
EBER	mRNA nuclear	Novocastra Leica Biosystems, UK	RTU	Proteinase K	Novocastra
Kappa	mRNA cytoplasmic	Ventana Medical Systems, Inc. Tucson, AZ, USA	RTU	Proteinase K	Ventana
Lambda	mRNA cytoplasmic	Ventana Medical Systems, Inc. Tucson, AZ, USA	RTU	Proteinase K	Ventana

HIER heat-induced epitope retrieval, *IHC* immunohistochemistry, *ISH* in situ hybridisation, *RTU* ready to use

slipping with Vectashield fluorescence mounting media (Vector Laboratories Inc, Burlingame, CA, USA) and evaluation with a Nikon Eclipse E1000 microscope equipped with a spectrum-green, orange, aqua, DAPI and triple band filter. Slides were scored after counting at least 100 nuclei. Cut-off values for the FISH analysis were 5 % for the BA probe and 15 % for the fusion probe.

Results

A total of 39 NHL cases conformed to the inclusion criteria. Twenty-three cases were confirmed as HIV positive and 16 were strongly suspected to be HIV positive on clinical findings. CD19, CD79a and bcl6 were not regarded as specific enough to reliably discriminate B cells from plasmablasts and PCs (see later). Cases were subdivided into two groups according to their staining for the additional B cell markers CD10 and Pax5. Statistical analysis was done using the Fisher exact test. Immunohistochemical characterisation of the lymphomas is summarised in Table 2. Group 1 ($n=23$) consisted of cases negative for both CD10 and Pax5, and group 2 ($n=16$) consisted of cases positive for CD10 and/or Pax5. Only four of the 16 cases (25 %) in group 2 were positive for both CD10 and Pax5, seven cases (44 %) were positive for only CD10 and five cases (31 %) were only Pax5 positive without CD10 reactivity. All cases showed a high Ki67 proliferation fraction (bordering on 100 % in some cases). No correlation could be found between morphology and the expression of additional B lineage markers. The presence of morphologically mature PCs varied but always constituted less than 1 % of the tumour cell population. CD38 expression was significantly higher in lymphomas of group 2 ($p=0.006$), whilst CD138 ($p=0.006$) and CD117 ($p<0.001$) expression was significantly higher in group 1. Interestingly, group 2 lymphomas also showed a much higher incidence of *MYC* gene breaks ($p=0.006$) and $t(8;14)$ rearrangements ($p<0.001$), than group 1. There were no statistically significant differences in the expression of LCA ($p=0.469$), bcl6 ($p=0.303$), CD79a ($p=0.363$), CD56 ($p=0.522$), kappa and lambda light chain restriction ($p=0.075$) or EBV EBER ($p=0.604$) between the two groups.

Discussion

NHLs are AIDS-defining neoplasms diagnosed with a high frequency in South Africa [3, 4]. The vast majority of HIV-related NHLs are aggressive, high-grade B lineage

Table 2 Comparison between cases with and without reactivity for Pax5 and CD10 as additional B cell markers

	Group 1 Negative for additional B-cell markers ($n=23$)	Group 2 Positive for additional B-cell markers ($n=16$)	Fisher exact
CD20	None	None	
CD45	13 (57 %)	8 (50 %)	$p=0.469$
CD3	None	1 (6 %)	
MUM1	23 (100 %)	16 (100 %)	
Blimp1	23 (100 %)	16 (100 %)	
BCL6	7 (30 %)	7 (44 %)	$p=0.303$
CD10	None	11 (69 %)	
CD19	None	2 (13 %)	
CD79a	1 (4 %)	2 (13 %)	$p=0.363$
Pax5	None	9 (56 %)	
CD38	10 (43 %)	14 (88 %)	$p=0.006$
CD138	20 (87 %)	7 (44 %)	$p=0.006$
CD56	4 (17 %)	2 (13 %)	$P=0.522$
CD117	2 (9 %)	None	
EBER	19/21 (90 %)	15/16 (94 %)	$p=0.604$
HHV8	None	None	
Light chain restriction	16/22 (73 %)	6/14 (43 %)	$p=0.075$
MYC BA	7/20 (35 %)	11/13 (85 %)	$p=0.006$
$t(8;14)$	3/17 (18 %)	9/10 (90 %)	$p<0.001$

lymphomas that demonstrate morphologic and immunophenotypic features suggestive of plasmablastic differentiation in only some of the tumour cells or diffusely throughout the neoplastic proliferation. Plasmablastic differentiation is loosely defined on the basis of the acquisition of morphological and immunophenotypic features portraying cellular commitment to the PC programme. Morphologically, plasmablasts are either large immunoblast-like cells with prominent central nucleolus in an eccentrically located nucleus or have multiple nucleoli in a slightly irregularly shaped nucleus. A combination of these morphological patterns is common. Immunophenotypically, plasmablasts are cells which have lost their B cell phenotype, acquired the transcriptional and antigenic profile of PCs and usually also demonstrate a high proliferation rate [5, 16, 43, 45]. Combinations of B cell and PC markers are therefore employed to define plasmablastic differentiation within a B lineage lymphoma. The presence of PRDM1/Blimp1, MUM1/IRF4 [28], and especially combined expression thereof are reliable indicators of this pathway of cellular differentiation.

According to the 2008 WHO Classification, PBL is no longer considered a subtype of DLBCL [45] but the differentiation between PBL and DLBCL, particularly if plasmablastic differentiation is present, remains problematic. Simplistically viewed, terminal differentiation of the B cell to

a PC necessitates shutdown of the B differentiation programme reflected by a loss of B cell markers and expression of PC differentiation markers such as CD38, CD138, CD56 and CD117. In reality, however, B cell to PC differentiation includes a less characterised transient phase represented by the plasmablast. The transient nature of a plasmablast, as reflected by its changing gene and protein expression, has prompted some investigators to sub-classify this phase into pre-plasmablast and plasmablast stages [27, 34, 47]. Progression between the different maturation stages is a gradual process with potential phenotypic overlaps between the late B cell and the early or pre-plasmablast as well as between the late or mature plasmablast and PC stages of development (Fig. 1). The exact antigen expression profile of neoplastic plasmablasts has also not been fully characterised and what is accepted to be in keeping with a diagnosis PBL varies amongst authors (Fig. 2). This may seem purely academic but unravelling these differences may become increasingly relevant in the era of targeted therapy.

Both the pre-plasmablast and plasmablast stages of B cell maturation were recently demonstrated to have complete absence of B cell antigen expression during normal maturation [27] (Fig. 2). Controversy however exists regarding the immunophenotype of neoplastic lymphocytes in this post-B cell phase of maturation. Weak expression of B cell markers by neoplastic plasmablasts in PBL is accepted by some authors [34, 41, 50] (Fig. 2), but this weak degree of positivity is not defined or quantified in these studies. This has thus introduced a significant degree of diagnostic confusion with respect to typing a neoplastic lymphoid proliferation as either a true case of PBL or DLBC with plasmablastic differentiation.

CD20 is one of the oldest and most commonly used B cell markers utilised in immunohistochemistry, but loss of CD20 does not exclude a B lineage and a more extensive antibody panel is required to confirm an apparent non-B cell lineage [12]. The panel of additional B cell markers in this study consisted of bcl6, CD79a, CD19, Pax5, and CD10. *BCL6* is acknowledged as a transcriptional repressor governing germinal centre (GC) formation [28, 52] but it is still unknown at which point in B cell maturation its upregulation occurs. Although downregulation of *BCL6* expression is necessary to complete the process of terminal differentiation to mature PCs, its presence does not inhibit initiation of PC differentiation [17] and a certain degree of remnant bcl6 positivity may therefore potentially be encountered in post-GC B cell neoplasms such as PBL. CD79a is a diagnostically sensitive pan-B cell marker expressed from the pro-B cell stage through to the PC stage. [32] The JCB117 clone utilised in this study was previously proven to exhibit the greatest degree of B cell lineage fidelity [32]. Interestingly, CD79a reactivity was seen in only three cases in this study consistent with results from previous studies [25, 50]. CD19, a transmembrane glycoprotein in the immunoglobulin superfamily [10] is regarded as

Fig. 1 The *dotted circles* in the diagram demonstrate the two phases in B cell maturation where potential phenotypic overlap in the expression of B cell and PC antigens may occur (*B* B cell, *PB* plasmablast, *PC* plasma cell)
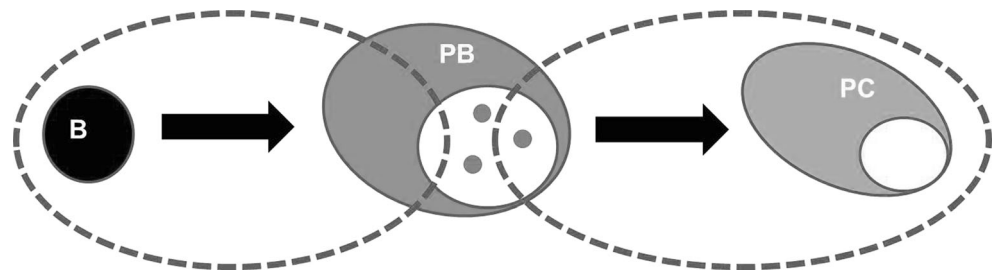
one of the most reliable surface biomarkers for B cells [51] and is expressed approximately at the time of immunoglobulin rearrangement through terminal differentiation to PCs [15, 51]. Interestingly, as with CD79a, CD19 reactivity was seen in only two cases, both of which also showed Pax5 positivity. The reasons why CD79a and CD19 are not expressed more often by these tumours of obvious B lineage is unknown and has not been addressed before. As with bcl6, the possibility of extended expression of both CD79a and CD19 into the post-GC/plasmablastic phases of B-cell differentiation warranted their exclusion as potential discriminating B cell markers in the quest to distinguish late-stage B cells from plasmablasts.

The two most promising additional B cell markers utilised for this study were Pax5 and CD10. Pax5 is a B cell-specific activator protein whose expression is largely limited to cells with B lymphoid lineage commitment. [38] It is regarded as a reliable B cell differentiation antigen before the cells acquire plasmacytic features. [12] Nine cases in this study showed weak to strong Pax5 positivity in most of the tumour cell nuclei. These cases all had clear and diffuse plasmablastic differentiation as defined by morphological features, a high proliferation index and strong, diffuse PRDM1/Blimp1 and

IRF4/MUM1 expression (Fig. 3). Whilst exceedingly rare, cases of PC myeloma showing aberrant Pax5 expression have been reported although these have usually also been CD20 positive [29]. Nonetheless, Pax5 expression remains a confirmatory marker of a B cell rather than plasmablastic or PC phenotype. CD10, a cell surface metallopeptidase is expressed on early lymphoid progenitors and a small subset of immature bone marrow B cells. CD10 expression is lost as the cells reach maturation and is re-expressed on proliferating B cells in which setting it is generally regarded as an early germinal centre (GC) B cell marker [7, 18]. CD10 is also useful in the diagnostic armamentarium for Burkitt lymphoma. [37, 45] Twenty to 40 % of DLBCL cases express CD10 [11] and are thereby recognised to have a clinically more favourable GC phenotype in risk-stratification algorithms [1, 24, 36]. In this study, six of the CD10-positive cases showed strong and diffuse membranous positivity in more than 80 % of the tumour cells, whilst five cases showed weaker expression in 20–50 % of their cells. Few studies relating to PBL have included Pax5 and/or CD10 staining in their immunohisto-chemical panels, but positivity for both markers has been reported in cases published as PBL [13, 14, 18, 50]. Although

Fig. 2 The diagram represents a summary of the antigen expression profiles of the pre-plasmablast (*pre-PB*) and plasmablast (*PB*) during maturation of a normal B cell as described by Jourdan et al [27]. As indicated by the two *red circles*, weak B cell antigen expression by abnormal/neoplastic plasmablasts is however accepted by some authors, causing diagnostic confusion when trying to differentiate between DLBCL with plasmablastic differentiation and true cases of PBL
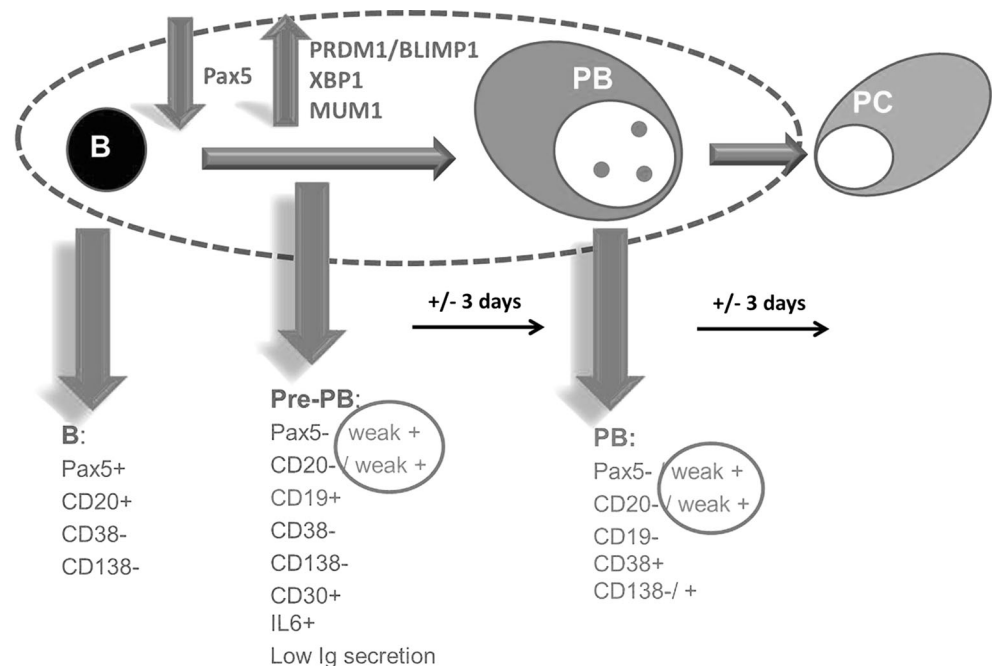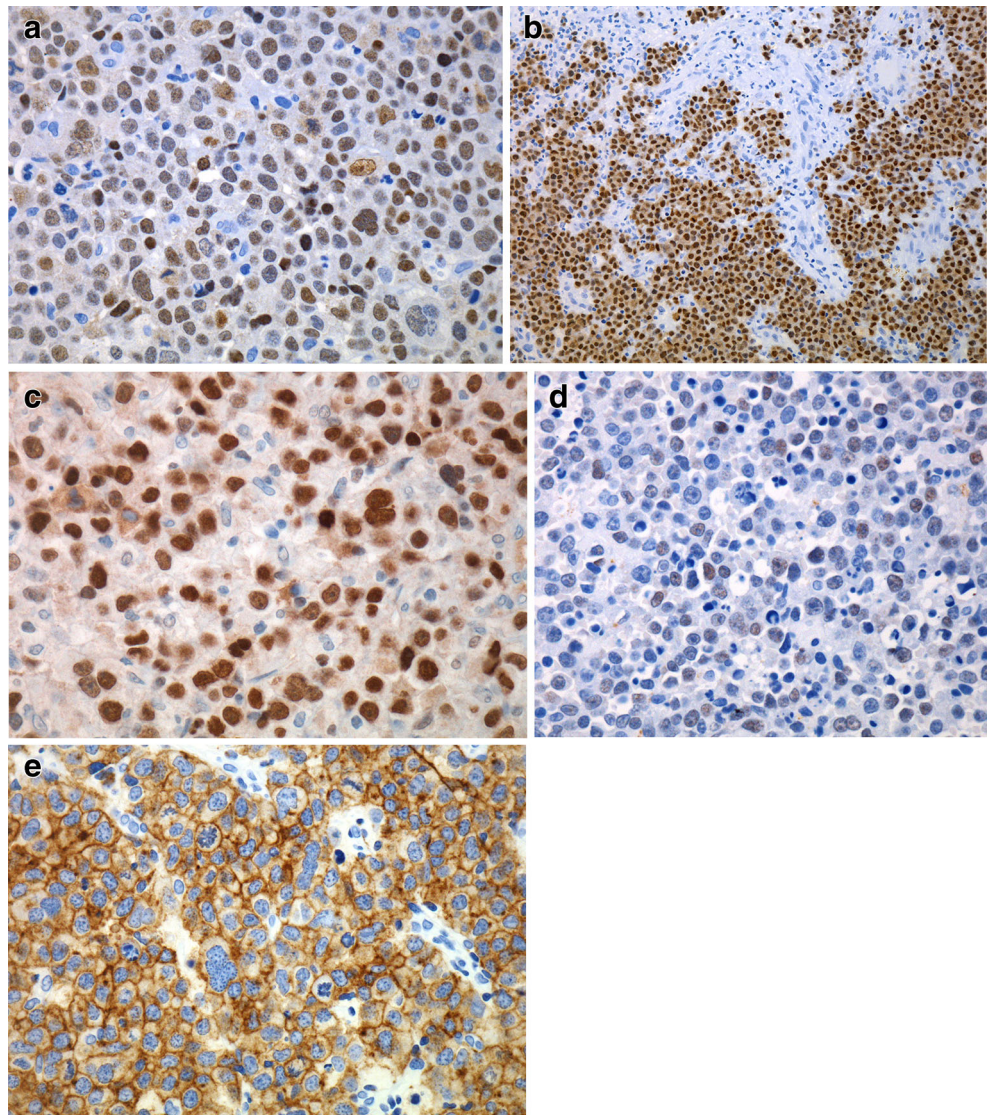
Fig. 3 These photomicrographs show a case with expression of the additional B cell markers in addition to MUM1/IRF4 and PRDM1/Blimp1 expression. **a** Diffuse Pax5 expression (score 0); **b** strong and diffuse MUM1/IRF4 and **c** PRDM1/Blimp1 stains of the same case. **d** An example of weak Pax5 expression (score 1). **e** An example of diffuse CD10 expression (score 0)
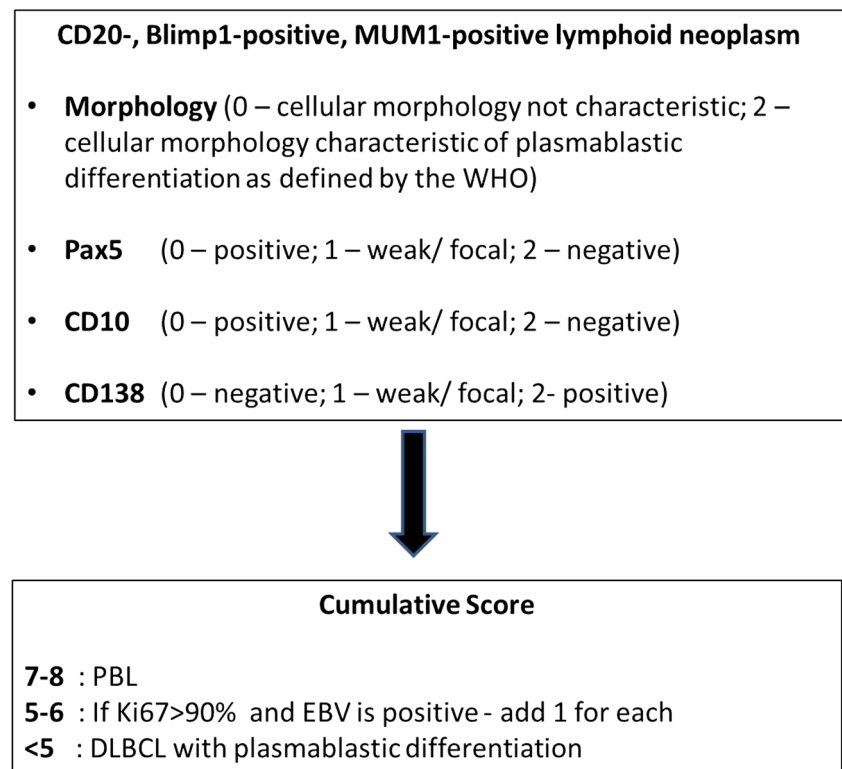

aberrant protein expression is possible and well recognised in neoplastic cells, the overt expression of early B cell markers such as Pax5 and CD10 (and especially both) in a neoplasm classified as plasmablastic lymphoma should be interpreted with great caution.

The use of PC differentiation markers in the diagnostic algorithm of PBL was also considered. The expression of CD38 and CD138 in our study population varied from completely negative to strongly and diffusely positive in 100 % of the tumour cells. CD38, a transmembrane glycoprotein is expressed on lymphoid as well as myeloid cells and used in the diagnostic armamentaria of PC myeloma [30] and Burkitt lymphoma [37]. In this study, CD38 was expressed significantly more often ($p=0.006$) in the group which showed additional B cell marker expression. The reason for this in uncertain but since CD38 positivity can be encountered in B cell and PC neoplasms, it was decided not to utilise this stain as a possible discriminator between B cell and PC stages

of development. CD138 is accepted as a universal marker of normal and malignant PCs [2] and was expressed significantly more commonly ($p=0.006$) in the B cell marker-negative group. The expression ratio of CD38 versus CD138 is also applied in the characterisation of the various stages of plasmablastic differentiation. [27, 34] In their flow cytometric study on normal plasmablast development, Jourdan et al. showed pre-plasmablasts to be CD20−, CD19+, CD38− and CD138−, plasmablasts to be CD20−, CD19+, CD38^{High} and CD138− and late plasmablasts/PCs to be CD20−, CD19−, CD38^{High} and CD138^{High} [27]. CD138, in contrast to CD38 which as alluded to before is regularly encountered in B cell neoplasms, seems to be more consistently associated with an advanced plasmablast or PC phenotype and was thus included in our diagnostic scoring system for PBL.

Other markers frequently used in the diagnostic armamentarium of PC neoplasms that were also applied in our study were CD56 and CD117. Both CD56 [33, 49, 19, 20, 42] and

Fig. 4 The diagram represents the summary of the proposed diagnostic scoring system to distinguish true cases of PBL from those high-grade NHLs which should rather be diagnosed as DLBCL with plasmablastic differentiation. Interpretation of scoring: *7–8*, consistent with a true case of PBL; *5–6*, PBL is not excluded. Supplemental Ki-67 and EBER testing recommended. A proliferation index of >90 % should add 1 point, and the presence of EBV another. If the score still remains <7, diagnose DLBCL with plasmablastic differentiation. If 7 or more, diagnose PBL. <*5*, DLBCL with plasmablastic differentiation

CD20-, Blimp1-positive, MUM1-positive lymphoid neoplasm

- **Morphology** (0 – cellular morphology not characteristic; 2 – cellular morphology characteristic of plasmablastic differentiation as defined by the WHO)

- **Pax5** (0 – positive; 1 – weak/ focal; 2 – negative)

- **CD10** (0 – positive; 1 – weak/ focal; 2 – negative)

- **CD138** (0 – negative; 1 – weak/ focal; 2- positive)

Cumulative Score

7-8 : PBL
5-6 : If Ki67>90% and EBV is positive - add 1 for each
<5 : DLBCL with plasmablastic differentiation

CD117 [2, 31, 39] are reported to be expressed by neoplastic PCs and CD56 is also positive in a small percentage of non-neoplastic PCs [6, 31]. Its role in B cell maturation and expression on plasmablasts have, however, never been investigated and its utility to distinguish between B cells, plasmablasts and PCs is thus not known. Previous studies have reported CD56 expression to range from 5 to more than 50 % in cases diagnosed as PBL [14, 26, 50]. Some ascribed this to aberrant expression in neoplastic plasmablasts [50] whilst others postulate it to be due to PBL arising secondary to dedifferentiation in a mature PC neoplasm [14]. Six cases (17.9 %) in this series showed CD56 reactivity in 40–100 % of their cells (Table 2) although no significant difference was noted between the two defined groups. Only two cases showed weak and focal expression of CD117 (Table 2) and were expectantly also negative for all the B lineage markers. Due to the uncertainty regarding expression patterns in these cells, these markers were excluded from the scoring system.

An interesting finding in this study was the significantly higher *t*(8;14) found in the PAX5/CD10-positive group lymphomas. The presence of MYC rearrangements, and specifically Burkitt types of MYC rearrangements, are generally more common in B cell malignancies derived from early stage of B cell development such as GC-derived DLBCL [35]. This is likely to reflect such DLBCL cases presenting with plasmacytoid features, a particularity described in HIV-associated lymphomas [23].

In this study, 92 % of all cases were positive for EBV EBER on in situ hybridisation. There is evidence that a pathogenic association may exist between EBV, HIV and a PC phenotype in B cell neoplasia. [8] Although EBV may be encountered in DLBCL with plasmablastic differentiation, this association is most striking in HIV-negative individuals. Thus, whilst EBV infection has been reported in 60 to 100 % of lymphomas diagnosed as PBL in the literature [4, 46, 48], its presence seems to be most valuable when attempting to distinguish PBL from DLBCL outside the HIV setting.

The proliferation fraction of PBL is known to be extremely high and borders on 100 % in many cases. Although EBV-positive DLBCLs may sometimes present with a proliferation fraction this high, a proliferation fraction of <80 % in a B cell lymphoma, especially in an HIV-negative patient, favours DLBCL over PBL. The proliferation fraction is therefore another useful (albeit less reliable) parameter in a PBL diagnostic scoring system such as this.

The current WHO categorisation of PBL as a distinct lymphoma entity and the recent evidence of a poorer outcome in PBL when compared to DLBCL make it imperative to differentiate true PBL from DLBCL with plasmablastic differentiation. No reliable criteria presently exist for reproducible distinction between these two lymphoma entities. Based on current knowledge and results from this study, we present a novel yet simple scoring system to distinguish true PBL from DLBCL with plasmablastic differentiation (Fig. 4). One of the strengths of the system is that it takes into consideration the

known variability in protein expression which may occur in PBL. Whilst additional validation of the scoring system is required and future, more specific markers may further refine this scheme, it represents an attempt to improve current diagnostic reproducibility of PBL amongst histopathologists. This is needed if we are to gain further insight and understanding into the oncogenesis and molecular biology of these enigmatic lymphomas.

Author contributions Prof SC Boy designed the research study, performed research, analysed the data and wrote the paper. Mrs MB van Heerden performed the research, analysed the data and assisted in the preparation of the paper. Prof R Pool analysed the data and assisted in the preparation of the paper. Dr P Willem analysed the genetic data and assisted in the preparation of the paper. Dr T Slavik performed the research, analysed the data and wrote the paper.

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