Aggressive large B-cell lymphoma with plasma cell differentiation: immunohistochemical characterization of plasmablastic lymphoma and diffuse large B-cell lymphoma with partial plasmablastic phenotype

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ABSTRACT

Background
Plasmablastic lymphoma has recently come to be considered a distinct entity among mature B cell neoplasms, although the limits with diffuse large B-cell lymphoma (DLBCL) need to be more accurately defined.

Design and Methods
Here we show the results of an immunohistochemical study of 35 cases of plasmablastic lymphoma compared with a set of 111 conventional DLBCLs .

Results
Our results demonstrate that the use of a limited combination of immunohistochemical markers (PAX5&CD20, PRDM1/BLIMP1 and XBP1s) enables the identification of a plasmablastic immunophenotype highly characteristic of plasmablastic lymphoma cases and associated with an aggressive clinical behavior. Additionally, the study shows that the acquisition of a partial plasmablastic phenotype (PRDM1/BLIMP1 expression) in DLBCL is associated with shorter survival in R-CHOP-treated patients.

Conclusions
The use of a restricted combination of immunohistochemical markers (PAX5&CD20, PRDM1/BLIMP1 and XBP1s) enables a more accurate definition of terminal differentiation for large B-cell lymphoma.

Key words: plasmablastic lymphoma, terminal B-cell differentiation.

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Introduction

Plasmablastic lymphoma (PBL) has recently come to be recognized as a distinct entity among mature B-cell neoplasms,[1] having formerly been classified as a clinicopathological variant of diffuse large B-cell lymphoma (DLBCL). It is thought to derive from terminally differentiated B cells, exhibiting an immunophenotype of plasma cells (PC).[2,3] Differential diagnosis with ABC-DLBCL or DLBCL with marked secretory differentiation[4] and plasma cell myeloma with plasmablastic morphology is still a common problem because of the lack of a distinctive phenotype.

Here we present the results of an immunohistochemical study of 35 cases of plasmablastic lymphoma, including cases of the oral mucosa type that closely match the initial description by Delecluse *et al.*,[5] and EBV-negative cases unrelated to immunosuppression and with clear-cut plasmablastic morphology. The study was performed in order to assess the phenotypic variability among tumors and to seek a specific immunohistochemical profile that would help to establish a differential diagnosis to distinguish this from conventional DLBCL. To this end, we have studied an additional larger cohort of DLBCL cases, investigating the correlation between the plasmablastic immunophenotype and clinical outcome. The study has taken advantage of the availability of new reagents for detecting plasma cell markers, such as XBP1s[6] and PRDM1/BLIMP1,[7] together with other well established markers.

Design and Methods

Thirty-five cases of diffuse large B-cell lymphoma with plasmablastic morphology and presence of plasma cell markers were retrieved from the CNIO (Madrid, Spain) consultation files. Their clinical features are summarized in Table 1. Cases diagnosed as large B-cell lymphoma associated with ALK expression, cavitary or extracavitary PEL, large B-cell lymphoma associated with multicentric Castleman disease and pyothorax-associated lymphoma were not included, although they commonly exhibit a plasmablastic phenotype which overlaps with that of these cases. It should also be noted that HHV8/LNA positivity by immunohistochemistry was an exclusion criterion in this series.

A set of 111 conventional DLBCL cases was also evaluated for comparison (see *Online Supplementary Table S1* for clinical features of the control series). The study protocol and sampling procedure were approved by the Carlos III Institutional Review Board. Informed consent was obtained when necessary.

Immunohistochemistry

Immunohistochemical staining was performed as follows: 2-4-µm-thick paraffin-embedded tissue microarrays (TMAs) and complete sections were cut onto Dako slides (DAKO, Glostrup, Denmark), and subsequently dewaxed, rehydrated and subjected to antigen retrieval by heating in 50 mM Tris [tris(hydroxymethyl)aminomethane] (Trizma base)-1 mM EDTA (ethylenediaminetetraacetic acid) (Sigma Chemical, St Louis, MO, USA) (pH 8) or citrate 10 mM pH 6.5 in a pressure cooker for 2 min. The slides were cooled and treated with peroxidase-blocking solution (DAKO) for 5 min.

Sections were then immunostained with antibodies against CD20, PAX5, BCL6, CD10, GCET1, KLHL6, IRF4/MUM1, PRDM1/BLIMP1, XBP1s, CD38, CD138, Ki67(MIB1) and p53. ISH for EBV-EBER (probe from VisionBioSystem Wetzlar, Germany) was also performed. (See *Online Supplementary Table S2* for details of the antibodies used and antigen retrieval methods.)

A set of 111 conventional DLBCL cases was also evaluated for comparison. All cases were reviewed beforehand and representative areas were selected. We used a tissue arrayer device (Beecher Instruments, Sun Prairie, WI, USA) to construct TMA blocks, according to conventional protocols.[8] Standard tissue sections were also analyzed when considered necessary.

Immunohistochemical evaluation was performed by two independent pathologists (ARGM and SMM). Disagreements were resolved by joint review on a multihead microscope. A uniform cut-off of 30% was adopted for all the markers used except p53 and Ki67. Cases with values below this threshold were considered weakly positive (+/-) if more than 10% of the cells were positive. Ki67 was quantified according to the percentage of positive cells at HPF magnification. P53 was semiquantified according to the intensity of the staining in the neoplastic population: mild, intermediate or high levels.

Statistics

The Kaplan-Meier method was used to estimate the distributions of overall survival (OS) and failure free survival (FFS).[9] Overall survival was considered as the time from diagnosis to the date of death from any cause or last contact. Failure free survival was calculated from the time of diagnosis to the date of relapse, death or loss. OS and FFS, both widely recognized clinical endpoints, were calculated according to the definition of Cheson *et al.*[10] The log-rank test was used to compare survival distributions.[11] SPSS version 15.0 (SPSS, Chicago, IL) was used for these analyses.

Results

Plasmablastic lymphoma has characteristic immunophenotypes

We found plasmablastic lymphoma cases to include two main immunophenotypes (Figure 1; Table 1).

The full plasmablastic phenotype consisted of loss of B-cell markers with the gain of two PC markers, PAX5&CD20-negative or weakly positive, PRDM1/BLIMP1-positive, XBP1s-positive (nuclear XBP1, spliced form) being found in 20 (57%) cases. The majority of them showed expression of surface CD138 (13 cases) (Figure 1A-C).

In addition to those cases originally diagnosed as plasmablastic lymphoma, 2 patients previously diagnosed as DLBCL (see below) showed the same full plasmablastic phenotypic profile with weak expression of PAX5&CD20 and co-expression of PRDM1/BLIMP1 and XBP1. In fact, a review of these 2 cases indicated that they showed an immunoblastic/plasmablastic morphology and are more appropriately classified as PBL (Table 1, cases 36 and 37).

A variant (faulty) plasmablastic phenotype (loss of B-cell markers, with fewer than two PC markers present) was observed in 15 (43%) cases, divided into: i) (PAX5&CD20-negative or weakly positive, PRDM1/BLIMP1-positive, XBP1s-negative) was seen in 11 cases (31%). Of these, only 5 showed expression of CD138 (Figure 1D and E); ii) atypical immunophenotypes: one case was PRDM1/BLIMP1-negative, XBP1s-positive, PAX5-negative; while 3 were PRDM1/BLIMP-negative, XBP1s-negative, PAX5&CD20-negative or weakly positive.

Interestingly, CD10 and two novel germinal center (GC) markers, GCET1 and KLHL6,[12] were present in a fraction of samples: 9 (26%) cases positive for GCET1, 5 (14%) cases positive for KLHL6 and 7 (20%) cases positive for CD10 (Figure 1C and E). Additionally, bcl6 was positive in 2 cases, which had been previously diagnosed as conventional DLBCL. IRF4/MUM1 was positive in all except 2 cases.

Differences in expression between EBV-positive and -negative cases were only significant with respect to the expression of B-cell markers CD20 and PAX5. Thus, 57% of EBV-negative cases were weakly positive for CD20 and 42% weakly positive for PAX5, while only 13% and 16% of EBV-positive cases were weakly positive for CD20 and PAX5, respectively; otherwise they were consistently negative. Ki67/MIB1 expression was uniformly high in all plasmablastic cases studied (mean 87%). P53 immunostaining showed weak/intermediate positivity in most of the cases, with 17% of them showing intense nuclear staining that was highly suggestive of p53 mutation.

Plasmablastic lymphoma phenotype is rare in conventional diffuse large B-cell lymphoma

These full and variant plasmablastic immunophenotypes are rare among conventional DLBCLs, including non-GC subtypes, according to Hans.[13] Specifically, while PRDM1/BLIMP1 positivity was found in 25% of the cases, positivity for nuclear XBP1s was almost absent (< 5% of cases) from the control series of DLBCLs. Only 5 out of 111 cases were positive for both PRDM1/BLIMP1 and XBP1s. A review of the HE slides and the immunohistochemistry profile of these DLBCL cases with PC markers revealed 2 of them to have weak CD20 and PAX5 positivity, while the other 3 were strongly positive for both B-cell markers. In fact, these 2 cases are better classified as PBL (Table 1, cases 36 and 37). It is of note that the remainder of the conventional DLBCL cases were clearly positive for CD20 and PAX5.

Clinical correlation: plasmablastic lymphoma and diffuse large B-cell lymphoma differ in their response to therapy and survival

Clinical features of the 35 PBL cases are summarized in Table 1. Most of the patients were male (21 males, 9 females, 5 unknown) and the median age was 48 years (range 31-84 years). The HIV status of 27 cases was investigated and 20 cases were found to be positive and 7 negative. Plasma cell myeloma was ruled out on the basis of clinical criteria (presence of lytic bone lesions, serum/urine

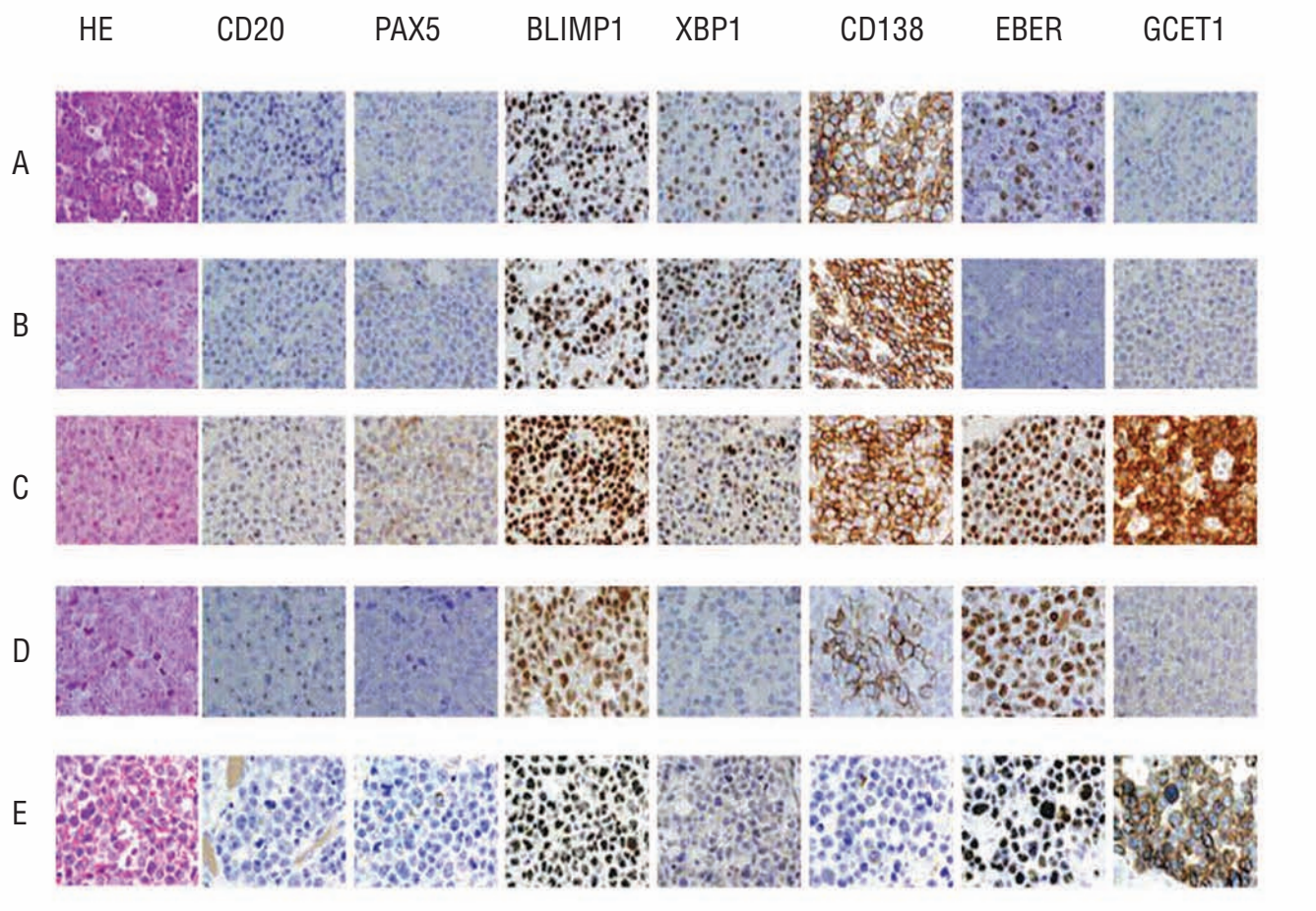

Figure 1. Distinct phenotypic profiles among plasmablastic lymphoma (PBL). (A) Full plasmablastic phenotype (PRDM1/BLIMP1-positive, XBP1s-positive, CD20 and Pax5-negative or weakly positive) with expression of CD138 and EBV-EBER (ISH). (B) Full plasmablastic phenotype in the absence of EBV-EBER. (C) Full plasmablastic phenotype with expression of GCET1. (D) Variant plasmablastic phenotype with expression of EBV-EBER. (E) Variant plasmablastic phenotype with expression of GCET1.

immunofixation or free light chain analyses). Bone involvement was observed in 5 cases, 4 of whom (cases 3, 4, 16, 18) showed sphenoid/maxillary bone involvement that fitted well with the tumor described as plasmablastic lymphoma of the oral cavity. The remaining case (case 28) showed multiple lytic bone lesions in the thoracic wall together with pleural cavity and lymph node involvement. Data regarding serum or urine paraproteinemia were absent after clinical history review.

Treatment-related data were available for 27 patients. Of these, 21 received CHOP or CHOP-like regimens. Seven of these 21 cases received combined immunochemotherapy with rituximab. Six patients did not receive chemotherapy because of very poor performance status or sudden death. Among the 21 patients who received chemotherapeutic regimens, 12 (57%) achieved complete remission (CR) after first-line treatment, 3 (14%) had a partial response (PR) and 6 (29%) did not respond (NR) or progressed.

Complete follow-up data were available for 28 patients. The median follow-up time for all these cases was ten months. The median follow-up among patients alive at last follow up was 23 months (range 5-61 weeks). The estimated 2-year OS was 42±10%; the estimated FFS was 42±12% (Figure 2). No significant differences were found in the estimated OS or FFS among patients who received anthracycline-based regimens with or without rituximab in this cohort of PBL cases (*data not shown*). The two cases reclassified as PBL featured an aggressive clinical behavior with failure of R-CHOP treatment by six and seven months, respectively.

Of the control group of 111 DLBCL cases, 58 (52.3%) were male and 53 (47.7%) were female (*Online Supplementary Table S1*). The median age was 62 years (range 23-88 years). Most of these patients received CHOP therapy (97 patients, 87%), 9 (8%) were treated with CHOP-like therapies and 5 (4.5%) with a MegaCHOP regimen. All patients received rituximab in combination with anthracycline-based regimens. CR was achieved in 88 (79%) patients, PR in 10 (9%) patients and NR/Progression

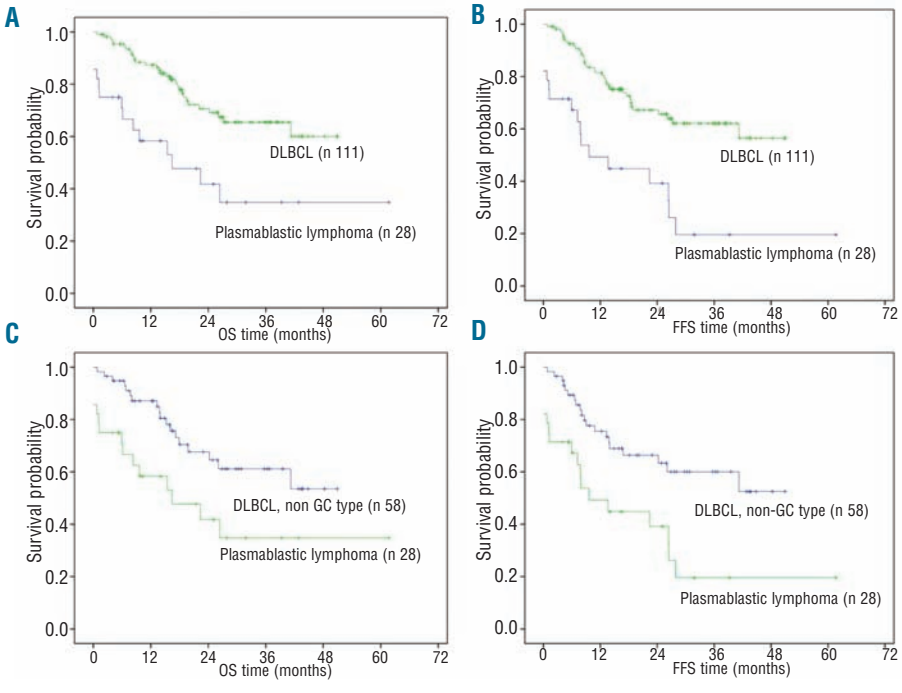

Figure 2. Plasmablastic lymphoma and DLBCL show clearly different responses to therapy and survival. (A and B) For OS and FFS, log-rank and Breslow tests plasmablastic lymphoma and conventional DLBCL survival curves were statistically significantly different (*P*<0.001). (C and D) In comparisons of non-GC type DLBCL and PBL, differences in OS and FFS were also significant (*P*<0.05).

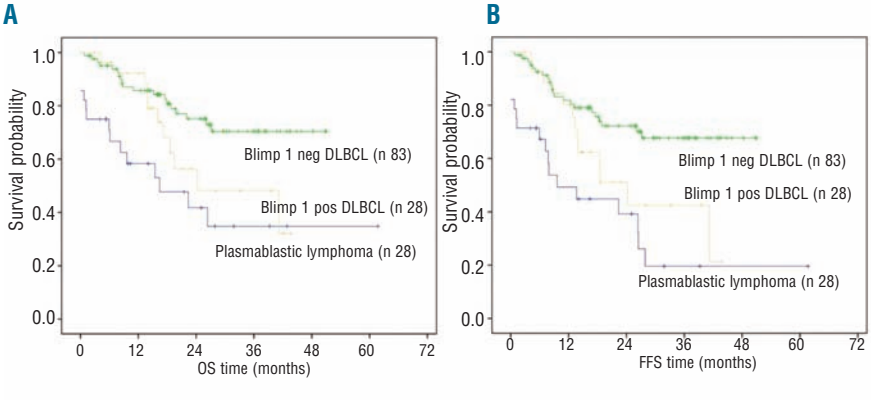

Figure 3. Overall (A) and failure free (B) survival curves for DLBCL divided taking into account PRDM1/BLIMP1 expression. A group of PRDM1/BLIMP1-positive DLBCL patients with prognosis intermediate between those of plasmablastic lymphoma and PRDM1/BLIMP1-negative DLBCL was identified. Estimated 2-year OS was 48±12%; estimated FFS was 44±12%.

in 11 (10%) patients. Response rates (patients achieving CR or PR) were significantly different between PBL and conventional DLBCL cases (χ^2 test, $P<0.05$).

Complete follow-up data were available for all patients in this group. The median follow-up time was 17 months (range 1-48 months). The median follow-up time among patients alive at last follow up was 23 months (range 2-48 months). The estimated 2-year OS was 69.0±5%; the estimated FFS was 65.7±5% (Figure 2). For both OS and FFS, log-rank and Breslow tests comparing PBL and conventional DLBCL survival curves were statistically significant ($P<0.001$) (Figure 2). Even when comparing PBL solely with non-GC type DLBCL,[13] the most aggressive subtype of DLBCL, differences in OS and FFS were significant: the estimated 2-year OS for non-GC DLBCL of 65 months ± 7% compared with 42 months ± 10% for PBL ($P<0.05$); the estimated 2-year FFS for non-GC DLBCL was 63±7% compared with 39±10% for PBL (Figure 2).

Diffuse large B-cell lymphoma with expression of PRDM1/BLIMP1

Additionally, when DLBCL cases were stratified according to PRDM1/BLIMP1 expression a group of patients with intermediate prognosis between PBL- and

Table 1. Phenotypic profile of PBL cases. Immunohistochemical scores are shown for PBL cases.

N	EBV-EBER (ISH)	CD20	PAX5	BLIMP1	XBP1	GCET1	KLHL6	BCL6	CD10	MUM1	CD38	CD138
1	+	-	-	+	+	-	-	-	-	+	+	+
2	+	-	-	+	+	-	-	-	+	+	+	+
3	+	-	-	+	+	-	-	-	-	+	+	+
4	+	+/-	+/-	+	+	-	-	-	-	+	+	-
5	+	-	-	+	+	+	+/-	-	+	+	+	-
6	+	-	-	+	+	+	-	-	-	+	+	-
7	+	-	-	+	+	-	-	-	-	+	+	-
8	+	-	-	+	+	-	-	-	-	+	+	-
9	+	-	-	+	+	-	-	-	-	+	+	+
10	+	+/-	-	+	+	-	-	-	-	+	+	+
11	+	-	-	+	+	+	-	-	-	-	+	+
12	+	-	-	+	+	-	-	-	+	-	+	+
13	-	+/-	-	+	+	-	-	-	+	+	+	+
14	-	+/-	+/-	+	+	-	+	-	-	+	+/-	-
15	+	-	+/-	+	+/-	+	+/-	-	-	+	+	-
16	+	-	-	+	+/-	+/-	-	-	-	+	+	+
17	+	-	-	+	+/-	-	-	-	+	+	+	+
18	+	-	-	+	+/-	+	+/-	-	-	+	+	+
19	+	-	+/-	+	+/-	-	-	-	-	+	+	+
20	+	-	-	+	+	-	-	-	-	+	-	+/-
21	+	+/-	+/-	+	-	-	-	-	-	+	-	-
22	+	-	-	+	-	+	-	-	-	+	+	-
23	+	-	-	+	-	+/-	-	-	-	+	+/-	+/-
24	+	-	-	+	-	-	-	-	-	+	+	-
25	+	-	-	+	-	-	-	-	-	+	+	+
26	+	+/-	+/-	+	-	-	-	-	-	+	-	-
27	-	+/-	+/-	+	-	-	+	+/-	-	+	+	-
28	-	-	-	+	-	-	-	-	+	+	+	+
29	-	-	-	+	-	-	-	-	-	+	+	+
30	+	-	-	+/-	-	-	-	-	+	+	+	+
31	+	-	-	+/-	-	-	-	-	-	+	+/-	-
32	+	-	-	-	+/-	-	-	-	-	+	+	+/-
33	+	-	-	-	-	+	+	-	-	+	+	-
34	+	-	-	-	-	-	-	-	-	+	+	+
35	-	+/-	+/-	-	-	-	-	-	-	+	+	-

Two well defined profiles are found: full plasmablastic phenotype (PRDM1/BLIMP1-positive, XBP1s-positive, Pax5 and CD20-negative or weakly positive) in 20 cases (57%) and variant plasmablastic phenotype (PRDM1/BLIMP1-positive, XBP1s-negative, Pax5&CD20-negative or weakly positive) in 11 (31%) cases. Four cases could not be classified by this particular combination of markers and were considered variant PBLs. Semiquantified scores: (+) if more than 30% of the neoplastic population stained positively for a given marker; (+/-) if more than 10% but fewer than 30% of the cells stained positively; (-) if fewer than 10% of the cells stained positively. NE, not evaluated.

PRDM1/BLIMP1-negative DLBCL was identified. These had an estimated 2-year OS of 42±10% and an estimated FFS of 39±10% (Figure 3). Most of these PRDM1/BLIMP1-positive DLBCLs were considered non-GC type according to the Hans algorithm (20/58 non-GC cases were PRDM1/BLIMP1-positive; 8/53 GC type cases were PRDM1/BLIMP1-positive; χ^2 test, $P<0.05$). These data demonstrate that the acquisition of a partial plasmablastic phenotype in DLBCL is associated with aggressive clinical behavior.

Discussion

Our results demonstrate a highly characteristic phenotype of PBL with simultaneous double expression of PRDM1/BLIMP1 and XBP1s together with absent or low levels of expression of B-cell markers (PAX5 and CD20). This particular profile is highly unusual in conventional DLBCL cases. In our control series, only 5 of 111 conventional DLBCL cases showed this particular combination. Following morphological and immunohistochemical review, it appeared that 2 of them may actually be PBLs (with immunoblastic/plasmablastic morphology, low levels of expression of the B-cell markers PAX5 and CD20 and short time to failure of R-CHOP therapy). The other 3 may belong to the category described as DLBCL with secretory differentiation[4] on account of their strong CD20 and PAX5 positivity.

In summary, this full plasmablastic phenotype (PRDM1/BLIMP1-positive, XBP1s-positive and CD20/PAX5-negative or weakly positive) helps to differentiate these tumors from conventional DLBCL. This plasmablastic immunophenotype, although more frequent in Epstein-Barr virus (EBV) -positive cases, also appears in EBV-negative cases. EBV has been demonstrated to play a role in the downregulation of the B-cell identity program through different mechanisms such as PAX5 promoter hypermethylation,[14] inducing at the same time ER stress-mediated XBP1s activation and nuclear accumulation.[15] We did not identify any alternative mechanisms for the EBV-negative cases showing a comparable phenotype, since HHV8-positive cases were not included. Additionally, we found that some EBV-positive cases failed to express XBP1s.

Some cases with plasmablastic morphology show what we have called a variant (faulty) plasmablastic phenotype, which may reflect different stages of the gradual terminal differentiation of B cells[16] (Figure 4). With regard to the correlation of the full and variant plasmablastic phenotypes with normal plasma cell differentiation it seems clear that in the neoplastic counterpart of the normal plasmablast, XBP1s expression is almost always accompanied by PRDM1/BLIMP1. Only one case showed a phenotype with positivity for XBP1s and negativity for PRDM1/BLIMP1 (case 32), an unusual combination that defines a differentiation stage.[16,17] Moreover, a full plasmablastic/plasma cell phenotype is not always found, even in mature plasma cell neoplasms, and additional data may be required to establish in greater depth the stages in the process of normal plasma cell differentiation.[18,19]

The discovery of these full and variant plasmablastic phenotypes is consistent with previous findings by Balague and co-workers concerning the expression of XBP1s in different lymphoma types.[18] These authors, using a polyclonal antibody against both cytoplasmic and nuclear XBP1s, reached similar conclusions to ours regarding the heterogeneity of XBP1s expression in PBL and the clinical impact of XBP1s expression in DLBCL. Differences in the proportion of XBP1s-positive DLBCL cases could be attributed to the use of different reagents; a monoclonal antibody that reacts only with the spliced form in this study,[6] and a polyclonal antibody in the study by Balague *et al.*[18]

In relation to the clinical relevance of the diagnosis of PBL, it seems clear in our series that this type of mature B-cell neoplasm behaves aggressively, responds poorly to therapy and has a short FFS and OS. Clear-cut differences from conventional DLBCL were found in response rates and survival. Even when compared solely with non-GC-type DLBCL, the most aggressive subtype of DLBCL, the differences in OS and FFS with PBL were significant (Figure 2).

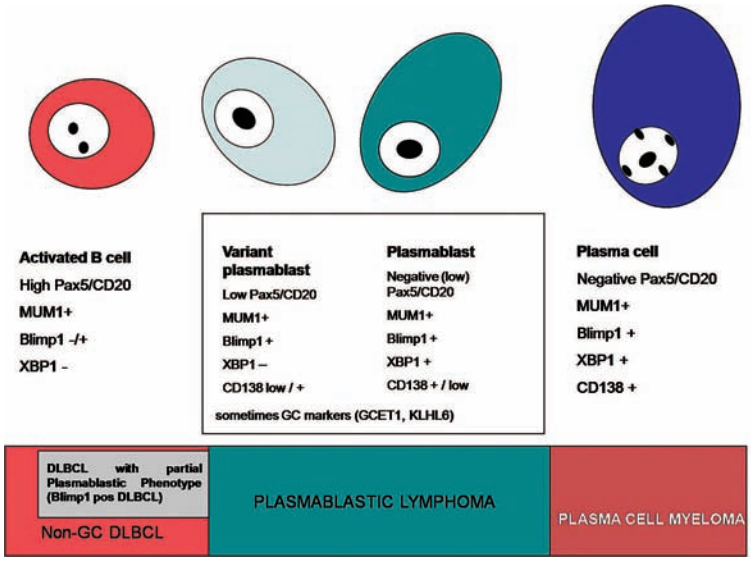

Figure 4. Use of a panel including PAX5&CD20, PRDM1/BLIMP1 and XBP1s allows a definition of a plasmablastic immunophenotype, which is variably expressed in PBL cases. The varying extent of this differentiation defines a spectrum of lesions that ranges from conventional DLBCL to plasmablastic lymphoma and identifies a group of aggressive large B-cell lymphoma tumors with immunophenotypic features intermediate between those of DLBCL and plasmablastic lymphoma.

Our data also demonstrate a relationship between the acquisition of a partial plasmablastic phenotype in DLBCL and clinical aggressiveness. When conventional DLBCL cases were stratified solely according to the immunohistochemical expression of PRDM1/BLIMP1, a marker of antibody-secreting cell differentiation and a functional repressor of bcl6 and PAX5,[20,21] a group of patients with intermediate prognosis between those of PBL and PRDM1/BLIMP1-negative DLBCL was identified (Figure 3). Although the expression of PRDM1/BLIMP1 is associated with the non-GC phenotype as defined by Hans *et al.*,[13] these PRDM1/BLIMP1-positive cases represent only a minority (28 cases; 34%), albeit a particularly aggressive one, of this subgroup of DLBCL cases in our series. The clinical prognostic value of these findings for DLBCL cases would need to be analyzed taking into account the recently created Choi algorithm,[22] but this goes beyond the scope of this article. Inactivation of *PRDM1/BLIMP1* genes has been found in a relatively high proportion of non-GC DLBCL cases by Pasqualucci and co-workers[23] in which plasma cell differentiation is presumably blocked. It is possible that DLBCL cases carrying PRDM1/BLIMP1 expression in this series represent mainly those DLBCL cases lacking PRDM1/BLIMP1 mutation or epigenetic inactivation.

It is of note that those rare DLBCL cases co-expressing PRDM1/BLIMP1 and XBP1s are part of the group of aggressive DLBCLs. The series also includes many cases that would have been considered to carry a variant PBL immunophenotype in the absence of strong CD20 and Pax5 expression.

The biological explanation for the poor response of PBLs to current therapies including immunochemotherapy with monoclonal antibodies against CD20 might be related to the partial or complete loss of surface B-cell markers[24] arising from the acquisition of the terminal differentiation program.[20,21] Additionally, loss of MHC II expression after induction of a terminal B-cell program,[21] with downregulation of CIITA by PRDM1/Blimp1,[25] could potentially be related to the adverse clinical outcome found in these cases, as described previously.[26,27]

Furthermore, this new transcriptional program, which is characterized in many cases by the overexpression of XBP1s[28] and its nuclear translocation, opens new therapeutic opportunities to proteasome inhibitors that destabilize the unfolded protein response.[29,30] Proteasome inhibition has been demonstrated to play a role in the therapy of DLBCL cases with an activated phenotype[31] and in cases of clear-cut PBL.[32] Additional genetic alterations could also have a role in the aggressive behavior found in PBL. Our data show that PBL cases have a very high proliferative index, which is consistent with the findings of Balague *et al.* of frequent C-MYC structural alterations, mostly t(8;14), in these PBL cases.[33] Furthermore, a significant proportion of cases show high levels of p53 protein as demonstrated by immunohistochemistry, suggesting genetic events affecting *p53* gene.[34]

Clinical correlation is occasionally required in the differential diagnosis of PBL and plasma cell myeloma with plasmablastic features[2] because of their nearly identical immunophenotypic profiles.[35] In our series, however, a significant percentage of PBL cases express novel GC markers (GCET1 and KLHL6)[12] that may facilitate this differential diagnosis and point to an origin in B cells that have experienced the germinal center reaction. A few cases of PBL with positivity for other germinal center B-cell markers, such as bcl6, have been described in the literature, suggesting that these cells may remain at an earlier (immunoblastic) stage of B-cell differentiation.[5,35] Interestingly, as our cases show, the phenotype of a particular case does not always fit perfectly with the hypothetical differentiation stage. In this sense, immunohistochemical expression of GCET1[12] can be found in tumors with a full plasmablastic phenotype, i.e. at late stages of terminal differentiation with both PRDM1/BLIMP1 and XBP1s expression. This expression of GC markers can be used as a diagnostic tool.

Although infrequent, another possible overlapping feature of plasma cell myeloma and PBL is the expression of CD20 by a small percentage of multiple myeloma cases.[19,36] Under these particular circumstances, however, CD20 expression by plasma cell myeloma is accompanied by a mature small cell morphology and t(11;14),[36] which are features absent from PBL.

In summary, the use of a restricted combination of immunohistochemical markers (PAX5&CD20, PRDM1/BLIMP1 and XBP1s) enables a more accurate definition of terminal differentiation for aggressive large B-cell lymphoma and provides an additional tool for the identification of this particular phenotype, which is highly characteristic of plasmablastic lymphoma cases according to the WHO classification.[1] Moreover, the use of this combination of markers allows the identification of a subset of poor-outcome DLBCL cases with features intermediate between those of DLBCL and PBL, who could be candidates for new treatment options.

Authorship and Disclosures

SM-M designed and performed the research, analyzed data and wrote the manuscript; ARG-M and SMR-P performed the research; LM and GR performed research and contributed vital reagents; LS-V performed research; MM, JFG, JM, CM, MCR-M and EC contributed clinical data; MAP designed the research and wrote the manuscript.

The authors have no conflicts of interest to disclose.

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