Diffuse blastoid B-cell lymphoma: a histologically aggressive variant of t(14;18)-negative follicular lymphoma

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Among the diffuse lymphomas of B-cell origin, we have encountered one variant displaying blastoid features that morphologically mimic lymphoblastic lymphoma, the blastoid variant of mantle cell lymphoma, and the so-called blastoid transformation of follicular lymphoma. To better characterize this entity, we studied eight cases morphologically, immunohistochemically, and by fluorescence *in situ* hybridization (FISH) for cytogenetic abnormalities commonly associated with follicular lymphoma and B-cell lymphomas exhibiting high-grade histological features. All eight cases were presented as *de novo* neoplasms, and displayed an entirely diffuse (five cases) or only minimal follicular (three cases) growth pattern. The neoplastic lymphoid cells were of medium size with round nuclei, fine chromatin, inconspicuous nucleoli, and high mitotic rate; they expressed CD10, BCL6, and BCL2—a phenotype consistent with follicle center cell origin. A proportion of cases expressed MUM1. Their lack of TdT and CYCLIN D1 distinguished them from lymphoblastic lymphoma and the blastoid mantle cell lymphoma, respectively. The neoplastic lymphoid cells consistently expressed CD43 (seven of eight cases) and occasionally other T-cell-associated antigens, including CD5, CD7, CD8, and CD57. Although all cases overexpressed BCL2, t(14;18) was not detected in any of the five cases examined by FISH; instead, extra copies of chromosome 18 were found in four of five cases. Finally, other cytogenetic abnormalities, including structural abnormalities of *BCL6* (allelic loss/gain, rearrangement), monosomy 7, del(13)(q14), and *MYC* allelic loss, were frequently detected. The combination of a B-cell CD10+ BCL6+ BCL2+ phenotype in the presence of structural abnormalities of *BCL6* is consistent with a follicular center cell derivation for our cases. The lack of t(14;18) seen in our cases, although rare in most cases of follicular lymphoma, has been nevertheless reported in cases of follicular lymphoma with a predominantly diffuse growth pattern. The molecular pathogenesis, clinical manifestations, and prognostic significance of these lesions remain to be elucidated.
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Diffuse lymphomas of B-cell origin represent a heterogeneous group of lymphoid neoplasms. The histologically aggressive subtypes of diffuse B-cell lymphomas include biologically different entities, such as B-lymphoblastic lymphoma/leukemia, Burkitt/Burkitt-like lymphoma, and diffuse large B-cell lymphoma. They also include subtypes of small B-cell lymphomas, which display blastoid morphology. The better-described subtypes are the blastoid variant of mantle cell lymphoma and the blastoid transformation of follicular lymphoma. Several of these variants are now recognized in the updated (2008) World Health Organization Classification.[1]

We have identified a group of diffuse B-cell lymphomas characterized by malignant cells that morphologically mimic those seen in blastoid variant of mantle cell lymphoma, blastoid transformation of follicular lymphoma, or B-lymphoblastic leukemia/lymphoma. We report here the clinical, morphological, immunohistochemical, molecular genetic, and cytogenetic findings of eight cases of diffuse B-cell lymphoma with blastoid morphology.

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These neoplasms are associated with a constellation of immunophenotypic and cytogenetic features that suggest that they may represent a distinct subtype within the spectrum of t(14;18)-negative follicular lymphoma.

In particular, we investigated the occurrence of cytogenetic events associated with this lymphoma subtype and, alerted by unexpected results of a case observed early in the study, we also evaluated for the presence of aberrant expression of T-cell-associated antigens in this unusual lymphoma subtype.

Materials and methods

Case Selection

Eight cases of diffuse blastoid B-cell lymphoma were identified in the consultation and institutional case files of the Division of Hematopathology in the Department of Pathology and Laboratory Medicine at the New York Presbyterian Hospital/Weill Cornell Medical College. Multiple 4-μm histological sections were prepared from formalin-fixed, paraffin-embedded tissue blocks for routine H&E evaluation, immunohistochemistry, PCR analysis, and fluorescence *in situ* hybridization (FISH). IRB approval was obtained for this study.

Immunohistochemical Methods

Immunohistochemical studies were performed on formalin-fixed, paraffin tissue sections using a TechMate 500 automated immunostainer (Ventana Medical Systems, Tucson, AZ, USA). The staining was performed according to a modified MIP protocol using the ChemMate ABC Peroxidase Secondary Detection System (Ventana Medical Systems) or the Cap-Plus Peroxidase Detection System based on the labeled streptavidin–biotin method (Zymed Laboratories, South San Francisco, CA, USA). Monoclonal and polyclonal antibodies to the following antigens were used for immunophenotyping of the cases: CD2 (AB75), CD4 (1F6), CD5 (4C7), CD7 (CD272), CD10 (56C6), CD16 (2H7), CD21 (2G9), CD23 (1B12), CD56 (CD564) (Novocastra Laboratories, Newcastle upon Tyne, UK); monoclonal rabbit CD3 (SP7), CYCLIN D1 (SP4) (LabVision, Fremont, CA, USA); CD8 (C8/144B), CD20 (L26), BCL2 (124), BCL6 (PG-B6), MUM1 (MUM1p) (Dako Cytomation, Carpinteria, CA, USA); CD57 (Leu-7) (BD Biosciences, San Jose, CA, USA); CD43(L60), Pax5(24) (BD Pharmingen, San Diego, CA, USA); TIA-1(26gA10F5) (Coulter Immunotech, Marseille, France); Ki-67(7B11) (Zymed Laboratories); P53(1801) (BioGenex, San Ramon, CA, USA); and polyclonal rabbit anti-TdT (Supertechs, Bethesda, MD, USA). Before staining, the sections were pretreated in a pressure cooker using 10 mM citrate buffer, pH 6.0 (TdT, CD2, CD3, CD4, CD5, CD7, CD10, CD16, CD20, BCL2, BCL6, CD57, Pax-5, Ki-67, and P53), or in a water bath using Dako Target retrieval solution high pH at 95°C for 40 min (CD56, TIA-1, and CYCLIN D1), or were stained without any pretreatment (CD43). Positive controls of tonsils or thymus (TdT), or non-Hodgkin's lymphoma expressing P53, and negative controls with mouse or rabbit IgG were run in parallel. Immunoreactivity was defined as follows: negative (<10% positively stained neoplastic lymphoid cells); weakly positive (10–30% positively stained neoplastic lymphoid cells);or positive (>30% positively stained neoplastic lymphoid cells). Ki-67 results were expressed as the percentage of positively stained nuclei of neoplastic lymphoid cells.

Polymerase Chain Reaction

PCR was performed on all eight cases of diffuse blastoid B-cell lymphoma using DNA extracted from three 4-μm tissue sections prepared from formalin-fixed, paraffin-embedded blocks, using the method previously published by Wright and Manos.[2] Amplification of the immunoglobulin heavy chain gene from both framework 2 and framework 3 part of the V segment to the J region was carried out using seminested PCR protocols, as described by Diss *et al*.[3] The T-cell receptor gamma (TCR-γ) chain gene was amplified using the method described by McCarthy *et al*.[4] After PCR amplification, 10 μl of each PCR product was run on 10% polyacrylamide mini-gels for 1 h at 125 V, which were then stained with ethidium bromide and viewed under UV light.

Fluorescence *In Situ* Hybridization

Interphase FISH analysis was carried out using standard *in situ* hybridization protocols on paraffin tissue sections from all eight cases. Commercially available probes (Vysis, Downers Grove, IL, USA) used for the detection of chromosomal abnormalities were as follows: LSI IgH (spectrum green)/BCL2 (spectrum orange) dual color, dual fusion translocation probe; LSI BCL6 dual color break apart rearrangement probe (spectrum orange 5′-LSI BCL6 and spectrum green 3′-LSI BCL6); CEP 7 (D7Z1) spectrum green probe; CEP 18 (D1871) spectrum green probe; LSI 13 (13q14) spectrum green probe; and LSI C-MYC (8q24.12–q24.13) spectrum orange probe. At least 100 nuclei, or in most cases 200 nuclei, were scored. A case was considered positive if at least 20% of the nuclei contained the chromosomal abnormality in question.

Results

Clinical Features

The clinical findings are summarized in Table 1. The eight patients ranged in age from 24 to 88 years (median 64.5 years), and included four males and

Table 1 Clinical features of eight cases of diffuse blastoid B-cell lymphoma

Case	Age (yrs)/ gender	Clinical features	Biopsy site	Stage	Treatment	Outcome
1	88/F	Inguinal LAD, disseminated disease	Inguinal LN	IV	Unknown	Unknown
2	67/F	Cervical LAD, pleural effusion	Cervical LN	III	CHOP	Alive, in CR 10 months
3	24/M	Cervical LAD	Cervical LN	I	Unknown	Unknown
4	64/M	Cervical and mediastinal LAD, subclavian vein thrombosis, neurological deficits	Cervical LN	III	Unknown	Unknown
5	65/M	Lytic lesion of left tibia, progressive ankle pain	Bone (tibia)	I	CHOP, Rituxan, then radiation	Alive, in CR 10 months
6	40/M	Left axillary and infraclavicular mass	Axillary LN	II	CHOP, Rituxan	Alive, in CR 13 months
7	57/F	Posterior cervical LAD	Cervical LN	I	CHOP, Rituxan, then radiation	Alive, in CR 17 months
8	80/F	Multiple mediastinal LAD, thrombocytopenia	Mediastinal LN	III	CHOP, Rituxan	Died of disease 8 months

CHOP, cyclophosphamide, vincristin, adriamycin, and prednisone; CR, complete remission; LAD, lymphadenopathy; LN, lymph node.

four females. All patients presented with *de novo* lymphoma manifesting as either lymphadenopathy (seven cases) or a lytic bone mass (1 case). None were reported to have either bone marrow or peripheral blood involvement, and their clinical stage was heterogeneous. Clinical follow-up information was available for five patients, all of whom had received a CHOP (cyclophosphamide, vincristin, adriamycin, and prednisone)-based chemotherapeutic regimen. Four patients were also treated with Rituxan, two of whom were subsequently given radiation therapy. Of these five patients, four are alive and in remission, whereas one died of disease at the time of completion of this study.

Morphological Findings

The histological appearances of the eight cases were similar. The cases with nodal presentation displayed near total effacement of the nodal architecture. In five cases, the neoplastic proliferation exhibited an entirely diffuse growth pattern (Figure 1a). In three cases, focal areas accounting for ≤10% of the neoplastic proliferation displayed a follicular growth pattern (Figure 1b). Variable numbers of scattered epithelioid histiocytes and/or tingible body macrophages were seen in most cases, which imparted a starry sky appearance focally (Figure 1c). The neoplastic cells were medium in size, that is, nuclear size intermediate between that of a small lymphocyte and macrophage. The nuclei were regularly round and contained finely dispersed chromatin and one to multiple small nucleoli; they were surrounded by poorly defined cytoplasm (Figure 1d). Numerous mitoses were noted (5–16 per high-power field). Significant necrosis was seen in only one case (case 8).

Immunohistochemical Findings

The results of immunohistochemical studies performed on each case are summarized in Table 2. In all cases, the neoplastic infiltrate was comprised of B-cells, based on their expression of CD20 (Figure 2a) and Pax-5. The tumor cells from all cases were also immunoreactive for the follicle center cell-associated antigens, CD10 (Figure 2b) and BCL6 (Figure 2c), and overexpressed the BCL2 protein (Figure 2d). In four of the cases, the neoplastic cells expressed MUM1 (two cases strongly). In none of the cases did the neoplastic cells express CYCLIN D1 (Figure 2e) or TdT (Figure 2f), nor did they overexpress the p53 oncoprotein (Figure 2g). Seven of the eight cases of blastoid diffuse B-cell lymphoma expressed CD43 (Figure 2h). Of these, one (case 6) was also CD7 positive (Figure 3a, b) and CD57 positive, and three other cases expressed one additional T-cell marker each (case 1: CD57; case 4: CD5; case 5: CD8) (Figure 3c, d). In only one case (case 8), were the neoplastic cells devoid of T-cell antigens. In keeping with their blastic qualities, the tumor cells were associated with a high proliferation rate (80% or greater), based on semiquantitative determination of Ki-67 nuclear reactivity. In all cases, the proliferative pattern was diffuse or predominantly diffuse. CD21 allowed the demonstration of the presence of meshworks of follicular dendritic cells focally in three cases (the same cells expressed CD23 in only two cases).

Polymerase Chain Reaction

PCR was performed on all eight cases of blastoid diffuse B-cell lymphoma, of which DNA was amplifiable in six cases. All six cases showed clonal rearrangement of the immunoglobulin heavy chain gene, as shown by the framework 2 and/or framework 3 primers; none displayed rearrangement of the TCR-γ gene (Table 3).

Cytogenetic Analysis

FISH analysis was attempted in all eight cases and was successful in five cases of blastoid diffuse B-cell lymphoma. These results are summarized in Table 3.

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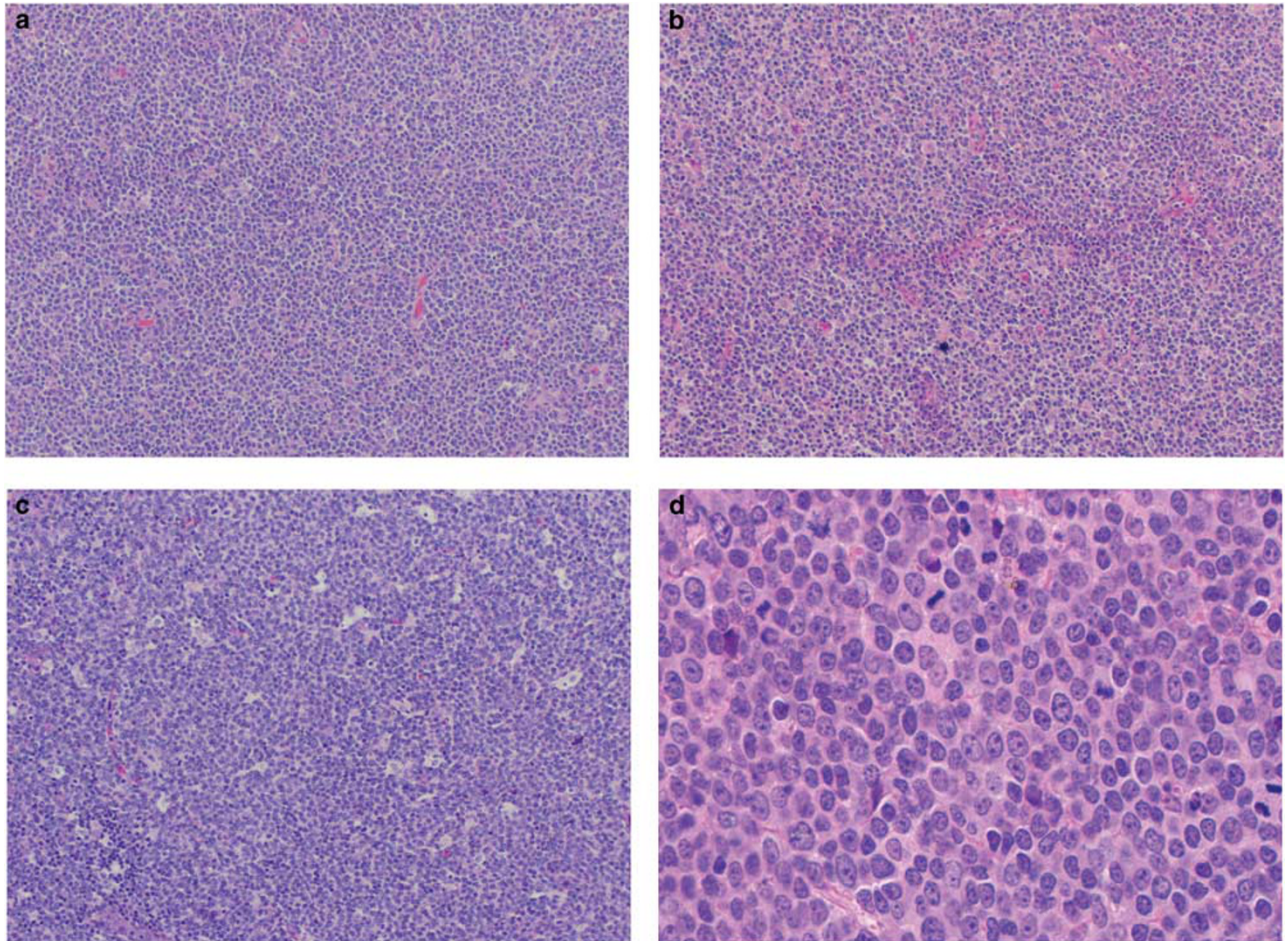
Figure 1 Morphological features of diffuse blastoid B-cell lymphomas. The architecture of this lymph node is effaced by an abnormal lymphoid proliferation with mostly diffuse (**a**; × 150), and only focal, vaguely nodular growth pattern (**b**; × 150). Scattered epithelioid histiocytes and/or tingible body macrophages are seen in most cases, which on low magnification impart a starry sky appearance (**c**; × 150). The tumor cells are medium-sized with round nuclei, finely dispersed chromatin, multiple small nucleoli, and high mitotic rate (**d**; × 600). These histological features are similar in all eight cases in this study.

Table 2 Immunophenotypic findings of eight cases of diffuse blastoid B-cell lymphoma

Case no.	CD20	Pax-5	CD21	CD23	CD10	BCL2	BCL6	MUM-1	CD43	CD2	CD3	CD5	CD7	CD4	CD8	TIA-1	CD16	CD56	CD57	Ki67	p53	TdT	CYCLIN D1
1	+	+	+	wk+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	wk+	>90%	−	−	−
2	+	+	−	−	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	90%	−	−	−
3	+	+	+	−	+	+	+	−	+	−	−	−	−	−	−	−	−	−	−	80%	−	−	−
4	+	+	−	−	+	+	+	+	+	−	+	−	−	−	−	−	−	−	−	>90%	−	−	−
5	+	+	−	−	+	+	+	+	+	−	−	−	+	−	−	−	−	−	−	>80%	−	−	−
6	+	+	−	−	wk+	+	+	−	+	−	−	+	−	−	−	−	−	−	+	>90%	−	−	−
7	+	+	+	+	+	+	+	−	+	−	−	−	−	−	−	−	−	−	−	>90%	−	−	−
8	+	+	ND	ND	wk+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	>90%	−	−	−

ND, not done; wk+, weakly positive.

None of the five cases showed t(14;18)(q32;q21), based on the lack of *IgH-BCL2* fusion signals. However, increased copy number of the *BCL2* gene was observed in four cases (Figure 4a), which resulted from an increased copy number of chromosome 18, as shown by the centromeric CEP 18 probe; the copy number ranged from three to six (Figure 4b). Two intact green signals, representing two copies of *IgH* gene, were seen in all cases. Four cases showed abnormalities involving the *BCL6* gene: these included loss of one allele (two cases), gain of one allele (one case), and rearrangement (one case). All five cases showed deletion of the 13q14 region, whereas four cases showed monosomy 7. None of the cases showed a *MYC* gene rearrangement; however, loss of one allele was observed in four cases.
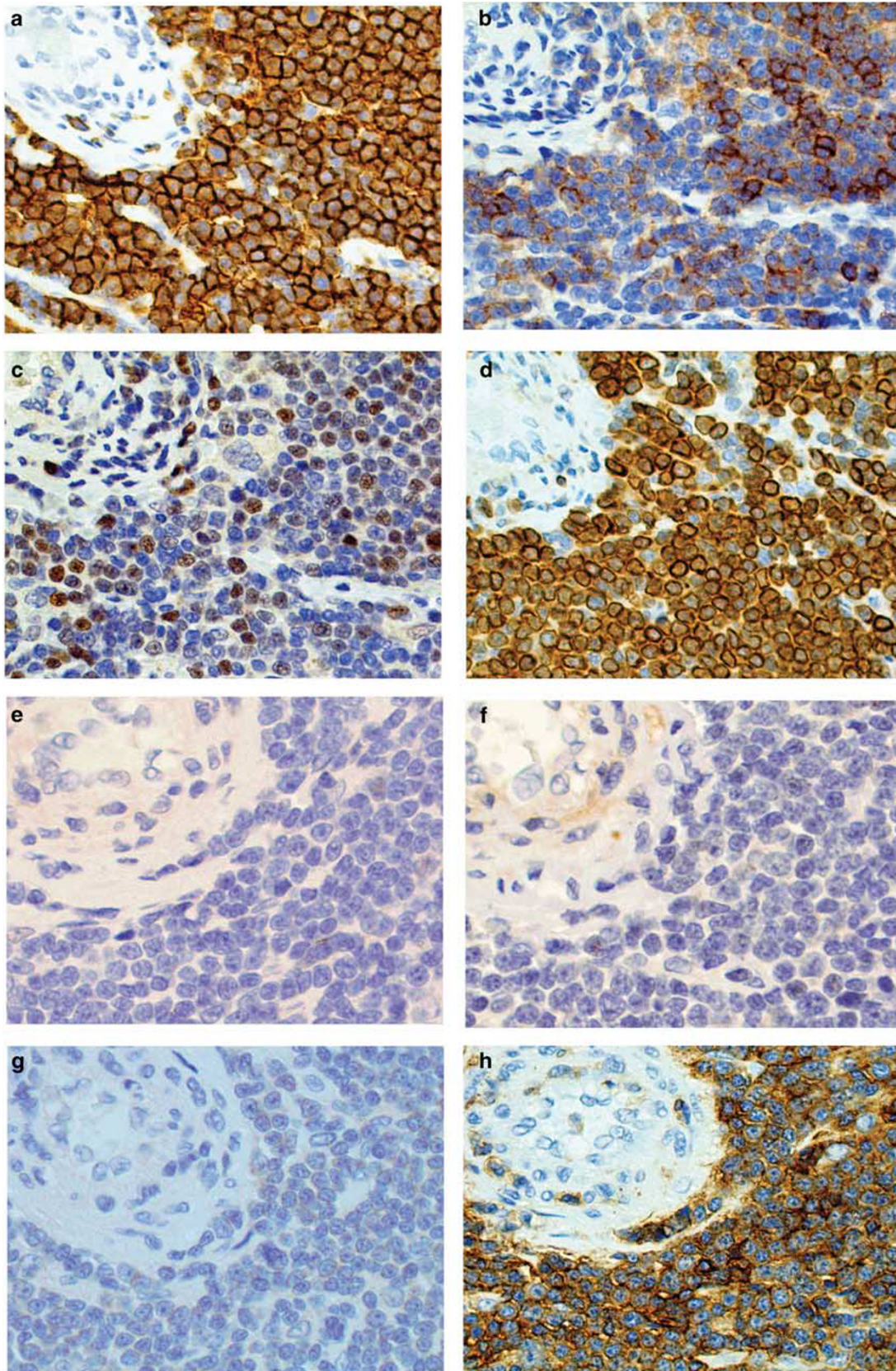
Figure 2 Immunophenotypic features of diffuse blastoid B-cell lymphomas. The neoplastic cells in all eight cases are strongly CD20 positive (**a**), express the follicle center cell-associated antigens CD10 (**b**) and BCL6 (**c**), and strongly overexpress BCL2 (**d**). However, immunostaining for CYCLIN D1 (**e**) and TdT (**f**) are negative, and the tumor cells do not overexpress the p53 oncoprotein (**g**). The vast majority of the cases show strong immunoreactivity for CD43 (**h**). Photographs were taken from case no. 4.
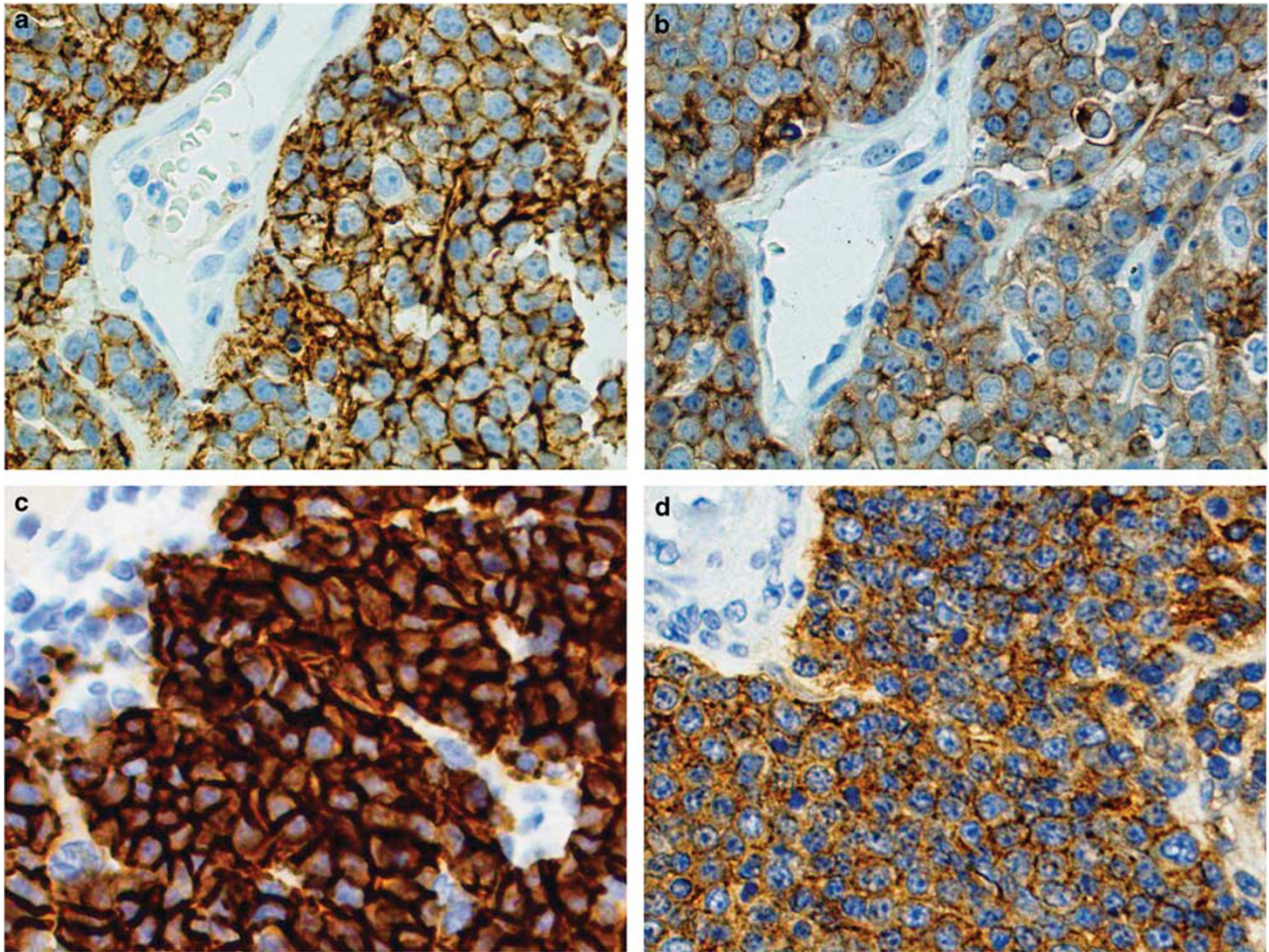
Figure 3 Aberrant T-cell-associated antigen expression. Although the B-cell origin of the tumor cells is shown by strong CD20 (**a**, **c**) expression, case no. 6 also shows aberrant expression for both CD7 (**b**) and CD57 (not shown), whereas case no. 5 also expresses CD8 (**d**).

**Table 3** Summary of PCR and fluorescence *in situ* hybridization results

Case no.	PCR		FISH						
	JH	Tγ	CEP7	LSI 13q	BCL6	MYC	t(14;18)	CEP18
1	R	G	Normal	del(13)(q14)	Allele loss	Allelic loss	—	Normal
2	R	G	del(7)	del(13)(q14)	Allele loss	Allelic loss	—	Four to six copies
3	R	G	del(7)	del(13)(q14)	Normal	Allelic loss	—	Four to six copies
4	R	G	del(7)	del(13)(q14)	R	Normal	—	Four to five copies
6	R	G	NS	NS	NS	NS	NS	NS
7	R	G	del(7)	del(13)(q14)	Allele gain	Allelic loss	—	Three copies

G, germline; NS: not successful; R, rearranged (detected by either framework 3 and/or framework 2 primers).
DNA for cases 5 and 8 was not amplifiable.

In summary, absence of t(14;18)(q32;q21) (all five cases), increased the copy number of *BCL2*/chromosome 18 (four cases), *BCL6* abnormalities (four cases), del(13)(q14) (all five cases), monosomy 7 (four cases), and loss of one *MYC* allele (four cases) were observed in our series of blastoid diffuse B-cell lymphomas.

Discussion

We report eight cases of a histologically aggressive variant of B-cell lymphoma with diffuse or predominantly diffuse pattern and blastic morphology that possesses characteristic pathological features. Four of the five patients with clinical follow-up data
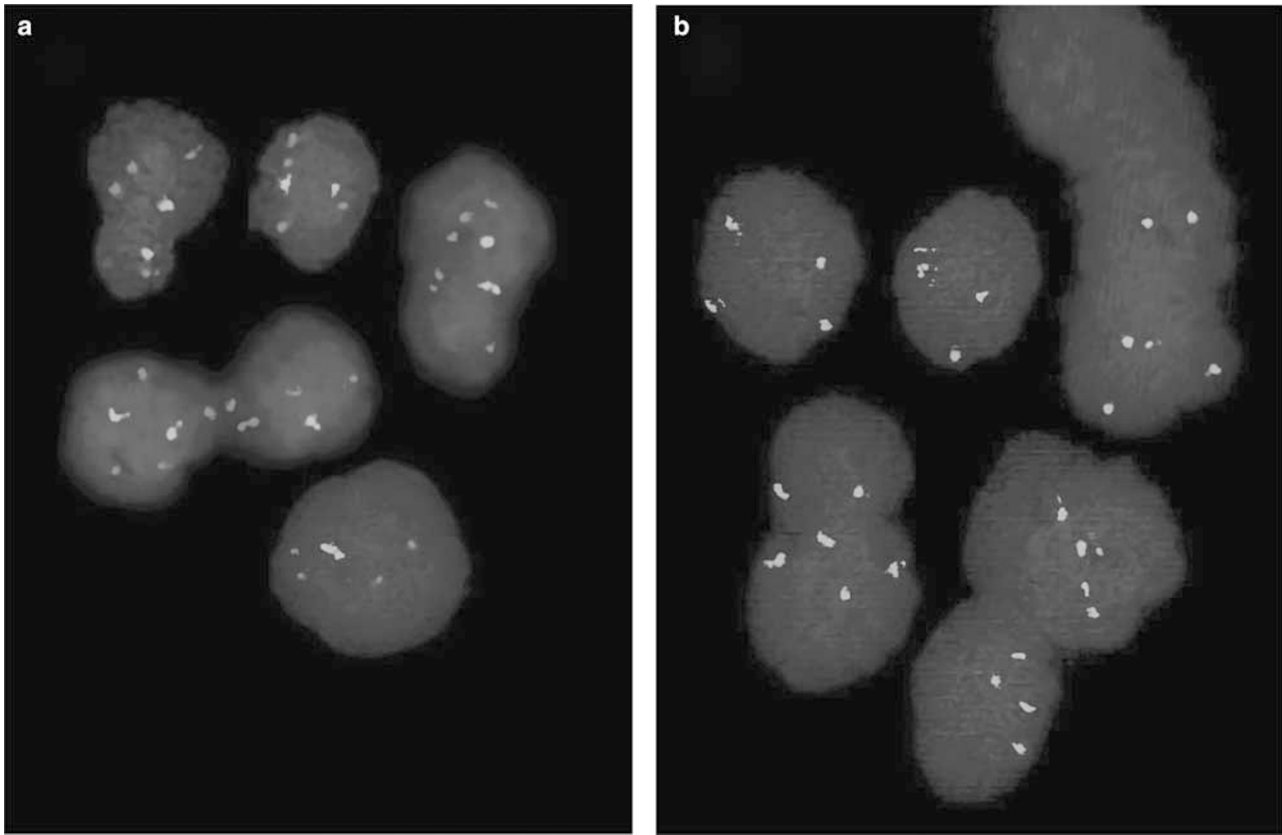
Figure 4 Interphase fluorescence *in situ* hybridization. Increased copy number of *BCL2* is shown by increased number of red signals (color version appears online) (**a**), whereas two intact green signals representing two copies of IgH gene are observed; probes used were LSI IgH (spectrum green)/BCL2 (spectrum orange). CEP 18 spectrum green-labeled probe shows increased copy number of chromosome (**b**).

responded well to the treatment and achieved clinical remission, whereas one died of disease. Histologically, these neoplasms are composed of tumor cells of medium size with blastic morphology, a diffuse or predominantly diffuse growth pattern, expression of the follicle center cell-associated antigens CD10, BCL6, and BCL2, and aberrant expression of one or more T-cell-associated antigens. Furthermore, they are associated with a high proliferation rate, and frequently show cytogenetic abnormalities, involving the *BCL6* gene, monosomy 7, or deletion of the 13q14 region. Despite their follicle center cell characteristics, all these neoplasms lack the t(14;18) translocation, but instead often show an increased copy number of chromosome 18 that could possibly account for BCL2 overexpression. Gain of chromosome 18q21 has been shown to be a frequent genetic abnormality in cases of t(14;18)-negative follicular lymphoma[5] and of diffuse large B-cell lymphoma.[6] Although some of the features of diffuse blastoid B-cell lymphoma overlap with those of diffuse follicular lymphoma,[1,7] their cytological characteristics, such as the finely dispersed nuclear chromatin in nuclei lacking evident nucleoli and poorly defined cytoplasm, the expression of CD43, and their high proliferation rate, distinguish them from diffuse follicular lym-

phoma. The morphology and immunophenotype, and above all the lack of *MYC* rearrangement exclude Burkitt and Burkitt-like lymphoma. It is very difficult, based on cytology alone, to distinguish the tumor cells of diffuse blastoid B-cell lymphoma from those of lymphoblastic lymphoma or the blastic/blastoid variant of mantle cell lymphoma. However, lymphoblastic lymphoma and the blastoid variant of mantle cell lymphoma can be excluded in our cases by their lack of TdT and CYCLIN D1 expression, respectively. In addition, the immunophenotype of diffuse blastoid B-cell lymphoma is distinctly different from that seen in cases of mantle cell lymphoma. This is particularly important in view of the possibility of CYCLIN D1-negative mantle cell lymphoma, a rare but nevertheless well-documented occurrence.[8] Such a diagnostic possibility could have been considered in case 4, particularly in view of its positivity with CD5. However, the presence, in this case, of positivity with CD10, *BCL6*, and MUM1 tend to exclude blastoid mantle cell lymphoma as an alternative diagnostic possibility.

All the diffuse blastoid B-cell lymphomas in our study expressed the follicle center cell-associated antigens, CD10 and BCL6. Although neither CD10 nor BCL6 is *per se* a specific marker for follicular

lymphoma, as their expression has been reported in a subset of T-cell non-Hodgkin's lymphomas,[9,10] their simultaneous expression by a B-cell neoplasm is generally accepted to be consistent with a follicle center cell origin. This is supported by findings of DNA microarray and tissue array studies, in which expression of both CD10 and BCL6 was found to be closely associated with diffuse large B-cell lymphomas with a germinal center B-cell-like gene expression profile.[11,12] Expression of MUM1, which was seen in a proportion of our cases, is also consistent with the interpretation that these blastoid cases share a common derivation with high-grade follicular lymphoma, which are also frequently MUM1 positive.[13,14] A most noticeable finding in this series is the lack of a t(14;18) translocation. Although considered as the hallmark of follicular lymphoma, the negativity for t(14;18), however, does not by itself exclude a diagnosis of follicular lymphoma. Approximately 15–25% of otherwise typical cases of low-grade follicular lymphoma are negative for t(14;18).[7,15,16] Moreover, follicular lymphoma grade 3, particularly the 3B subset in which the follicles are entirely composed of large cells, are also, as a rule, t(14;18) negative.[17–19] Furthermore, the existence of a t(14;18)-negative subset of diffuse follicular lymphoma has been recently well documented.[7] In view of the observed morphological, immunohistochemical, and molecular results, our cases, therefore, most likely correspond to a histologically aggressive (that is, blastoid) variant of t(14;18)-negative follicular lymphoma with a predominant diffuse proliferative pattern.

B-cell non-Hodgkin's lymphomas generally lack aberrant expression of T-cell antigens with the exception of CD5 and CD43.[18–20] Although CD5 expression is characteristic of chronic lymphocytic leukemia/small lymphocytic lymphoma and mantle cell lymphoma, it is rarely observed in other categories of B-cell non-Hodgkin's lymphoma.[21–23] CD43 expression, although relatively common in several subtypes of B-cell non-Hodgkin's lymphoma, is unusual in follicular lymphoma (3%).[24] However, CD43 expression is detected relatively often in cases of high-grade follicular lymphoma both with and without diffuse areas.[1] Expression of T-cell antigens other than CD5 and CD43 in B-cell non-Hodgkin's lymphomas is exceptional.[20,25,26] The diffuse blastoid B-cell lymphomas in our study were almost always CD43 positive (seven of eight cases); four of these cases also expressed one or two additional T-cell-associated markers: CD5 (case 4), CD8 (case 5), CD57 (case 1), and both CD7 and CD57 (case 6). This T-cell-antigen reactivity seems to represent aberrant expression, as all six cases with successful DNA amplification showed clonal rearrangement of the immunoglobulin heavy chain gene, but not of the TCR-γ chain gene. The prognostic significance of T-cell antigen expression in B-cell non-Hodgkin's lymphomas remains somewhat controversial. CD5 expression in some series of

extranodal marginal zone lymphoma[27,28] and *de novo* diffuse large B-cell lymphoma[22,23,29] has been associated with a clinically aggressive course and tendency for dissemination, although other studies have failed to support these findings.[30] Only one of five patients with clinical follow-up data in our study died of disease. Interestingly, but perhaps coincidentally, this is the only case without T-cell antigen expression.

Several cytogenetic abnormalities were frequently detected in our cases of blastoid diffuse B-cell lymphomas. In the absence of fresh tissue that could be used for conventional cytogenetics, as most of our cases were consultation material, we evaluated our cases for chromosomal abnormalities commonly associated with high-grade B-cell morphology, that is, trisomy 7, del(13)(q14), and *MYC* rearrangement, as well as t(14;18) and *BCL6* rearrangements by interphase FISH on paraffin tissue sections.

Trisomy 7 is a frequent finding (25%) in follicular lymphomas with t(14;18), in which it has been associated with high-grade morphology, a diffuse growth pattern, and an aggressive disease course.[31–34] Gain of chromosome 7 is also frequently detected in diffuse large B-cell lymphoma, especially in those that transformed from low-grade follicular lymphomas.[35] Instead of a gain, we detected a loss of chromosome 7 in four of five cases of diffuse blastoid B-cell lymphoma. Monosomy 7 is common in myelodysplastic syndrome and acute myeloid leukemia, particularly in therapy-related cases, in which it is considered as a poor prognostic factor.[36] Monosomy 7 also may be occasionally seen in acute lymphoblastic lymphoma/leukemia, but is not particularly associated with other subtypes of non-Hodgkin's lymphoma. Therefore, the presence of monosomy 7 is a unique finding; however, its significance in our cases, if any, is not clear.

Deletion of 13q14 is found quite commonly in hematological malignancies. It is the most frequent genetic aberration in chronic lymphocytic leukemia/small lymphocytic lymphoma,[37] but has also been documented in multiple myeloma,[38] and in non-hematological neoplasms of the head and neck[39] and prostate.[40] Although del(13)(q14) is associated with longer survival in chronic lymphocytic leukemia/small lymphocytic lymphoma,[41] it has been reported to predict an unfavorable outcome after chemotherapy in multiple myeloma.[42] del(13)(q14) has been associated with higher grade histology and is thought to represent a late cytogenetic event in follicular lymphomas.[34,43] We have shown this event in all five evaluable cases of diffuse blastoid B-cell lymphoma, but could not determine its prognostic significance.

Abnormalities of the *MYC* gene, although characteristic of Burkitt lymphoma, have also been documented in diffuse large B-cell lymphoma, especially among those occurring in extranodal sites.[44] In addition, a small subset of follicular lymphoma, along with t(14;18)(q32;21), have a

Burkitt-type translocation involving 8q24, that is, t(8;14)(q24;q32), t(8;22)(q24;q11), or t(2;8)(q12;q24); these 'double hit' cases are associated with blastic morphology, an aggressive disease course, and short survival.[34,45] We did not detect *MYC* translocation, but rather found a loss of one *MYC* allele in four of our five cases, a feature which distinguishes diffuse blastoid B-cell lymphomas from the above mentioned cases of follicular lymphoma.

Rearrangement of the *BCL6* gene is an important chromosomal aberration in B-cell lymphomagenesis. The translocation at 3q27 may involve the *IgH* locus at 14q32, but may also involve various other chromosomes, for example, t(3;22) and t(2;3), resulting in deregulation of the *BCL6* gene located at this breakpoint.[46,47] *BCL6* rearrangement is seen in 30–40% of diffuse large B-cell lymphoma, but in only 6–14% of follicular lymphoma. Among the latter group, it is particularly common in cases of grade 3B follicular lymphoma, with the highest frequency reported in cases associated with diffuse areas. Our results are, therefore, consistent with a follicular center cell-derived lymphoma, and suggest strong similarity to cases of 3B follicular lymphoma, although with a diffuse growth pattern and blastoid morphology.

Among the cytogenetic alterations that we have evaluated, possibly the most interesting is the one that was not detected. Remarkably, all five cases with successful FISH lacked the *BCL2/IgH* (t(14;18)) rearrangement, and yet expressed the follicle center cell-associated markers, CD10 and BCL6, and strongly overexpressed the BCL2 protein. There was no evidence of a variant translocation involving IgH and another partner chromosome, as two intact IgH signals were observed in all cases by interphase FISH analysis. On the other hand, four of five cases showed multiple copies (three to six) of chromosome 18. In the past few years, t(14;18)-negative follicular lymphoma has been well characterized.[5–7,16] This follicular lymphoma subset includes two main subtypes,[16] the first lacking BCL2 overexpression but having t(3;14) or another 3q27 structural alteration, indicating that *BCL6* deregulation may represent an alternate molecular mechanism leading to the development of follicular lymphoma. The second group shows strong BCL2 overexpression and extra copies of chromosome 18. Our cases bear close resemblance to this second group, in which BCL2 overexpression was not likely the result of a *BCL2/IgH* translocation but rather due to an increased dosage effect. This might represent the mechanism of neoplastic transformation of blastoid diffuse B-cell lymphomas from germinal center cells, along a molecular pathway distinct from that of classic follicular lymphoma. In case 1, which showed a normal copy number of chromosome 18, another undefined molecular mechanism might have given rise to *BCL2* deregulation.

Although there are similarities between our cases of diffuse blastoid B-cell lymphomas and the rare cases of follicular lymphoma in blastic/blastoid transformation reported by Natkunam *et al*.,[48] namely, the blast-like cytology, focal presence of follicle-like structures, and expression of follicle center cell markers, the differences are much greater. In contrast with our cases of diffuse blastoid B-cell lymphomas, all those cases were preceded by a follicular lymphoma, were predominantly follicular in pattern, and all had t(14;18), as shown either by cytogenetics or by PCR. However, in view of their similarities, one cannot exclude that at least a proportion of our cases may represent a t(14;18)-negative diffuse counterpart of blastoid follicular lymphoma.

In conclusion, the eight cases of diffuse blastoid B-cell lymphoma evaluated in this study are *de novo* diffuse B-cell lymphomas with blastoid morphology that need to be differentiated from other hematological malignancies with blastoid or blastic features. They seem to be of follicle center cell origin, but lack t(14;18), show CD43 antigen expression, have extra copies of chromosome 18, and possess other cytogenetic abnormalities. This combination of features suggests that diffuse blastoid B-cell lymphomas may represent a distinct variant within the spectrum of t(14;18)-negative follicular lymphoma. In this context, the presence of high proliferative activity, CD43 expression, and not uncommon MUM1 expression would suggest a similarity to cases of grade 3 follicular lymphoma, a subset which also often lacks a t(14;18) translocation. Recognition of these cases by other investigators and further study of large number of cases of this unusual histological variant should help to further elucidate their pathogenesis and their natural history.

Disclosure/conflict of interest

The authors declare no conflict of interest.

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