Diffuse large B-cell lymphoma with primary retroperitoneal presentation: Clinico-pathologic study of nine cases


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Summary
Diffuse large B-cell lymphoma primarily presenting in the retroperitoneum (PRLBCL) has been the object of occasional reports, all based on dated techniques.

Materials and methods: Nine PRLBCLs – with clinical information and paraffin blocks available – were reviewed on morphologic, immunohistochemical and molecular grounds.

Results: At microscopic examination, the cases were characterized by a diffuse proliferation of large cells (CD20+, CD79a+, CD3-), displaying a wide rim of cytoplasm (clear in seven instances and acidophilic in two), associated with sclerosis and frequent compartmentalization. Phenotypic and molecular analyses showed that: a) three cases were bcl-2+, bcl-6+, HLA-DR+, and CD10+ (1/3), with associated follicular dendritic cell (FDC) component and bcl-2 gene rearrangements; b) four cases were bcl-2, bcl-6, HLA-DR, CD10, FDC, and bcl-2 gene rearrangement negative; c) two cases had border-line characteristics (bcl-2+, bcl-6+, FDC+, HLA-DR-, CD10-, and bcl-2 gene rearrangement+). The first subgroup was thought to be of follicular derivation, as was the third due to bcl-6 and FDC stains. Of the corresponding five patients, three are in complete remission and two died of disease within 12 months. No obvious, normal counterpart was detected in the remaining four tumors: the corresponding patients died of disease in 3–23 months. The problem of similarities between PRLBCL and primary mediastinal LBCL is discussed.

Conclusions: Although the present series is small, our findings suggest that PRLBCL may represent a more heterogeneous group of tumors than previously thought, which merits further phenotypic and molecular studies to broaden the understanding of its histogenesis and behavior.

Key words: clinics, immunohistochemistry, large B-cell lymphoma, molecular biology, retroperitoneum

Introduction
Malignant lymphomas primarily arising in the retroperitoneum have been the object of only a few reports [1–4]. To the best of our knowledge, the largest series on retroperitoneal lymphomas was published by Waldron et al. in 1983 [4]. On the basis of pure morphology, these authors claimed that 74% of retroperitoneal lymphomas were sclerosing variants of follicle center cell lymphoma (FCCL), 14.7% immunoblastic lymphomas, 7.3% FCCLs without sclerosis, and 4% Burkitt’s lymphomas [4]. Of the 22 FCCLs with sclerosis reported by Waldron et al. [4], five were classified as small-cleaved cell lymphomas, 14 as large-cleaved cell tumors, and three as large non-cleaved cell forms. In all instances, the growth was follicular or follicular and diffuse, and was associated with marked sclerosis with compartmentalization [4]. In 19 out of 22 cases, lymph nodes – never displaying intraparenchymal sclerosis – were present within the biopsy material. The patients were predominantly females with an average age of 59.5 years and presented with abdominal mass and pain [4]. Infiltration of the mesenteric root was observed in ten cases. Eleven cases were stage I or II, the remaining ones being III or IV: all examples of large non-cleaved cell lymphoma had stage IV disease [4]. Half the patients achieved complete remission and six died of disease (i.e. all subjects with large non-cleaved cell lymphoma, who were also non-responders, one patient with large cleaved cell lymphoma, who achieved only partial remission, and two individuals with small cleaved cell tumor, who had partial remission and relapsing disease respectively) [4].

Herein, the authors report on nine examples of diffuse large B-cell lymphoma (DLBCL) [5–7] with primary presentation in the retroperitoneum (PRLBCL): their clinical, morphologic, phenotypic, and molecular findings are discussed and tentatively compared with those of primary mediastinal LBCL (PMLBCL) [8–34].

Materials and methods
Case selection
Nine DLBCLs, which primarily presented in the retroperitoneum in the absence of mediastinal involvement, were retrieved from the files
of the Unit of Pathology and Hematopathology of Bologna University. Formalin-fixed, paraffin-embedded tissue blocks [35, 36] were available, which corresponded to samples obtained either by laparotomy or ACT-guided needle biopsy (in seven and two cases, respectively). Clinical information included: age, sex, sites of disease, stage, presence of systemic symptoms, bulky disease, outcome, and therapies administered.

**Histological and immunohistochemical methods**

Three-micron-thick sections were cut from the paraffin blocks and stained with hematoxylin and eosin (H&E), Giemsa, periodic acid-Schiff (PAS) with and without diastase digestion, and Gomori silver impregnation for reticulin fibers [35, 36]. Further sections were cut, coated on naturally charged slides, stored in a warm chamber at 56 °C for at least two hours and then rinsed with water through repeated washes in Bioclear and graded alcohols. These sections were used for immunohistochemistry, which was performed by applying the panel of antibodies listed in Table 1; the latter were dispensed on a TechMate 500 immunostainer and detected by the alkaline phosphatase-anti-alkaline phosphatase (APAAP) technique [37-40]. Antigen retrieval was performed according to reported protocols [39]. In all instances, positive and negative controls were used in order to assess the reliability of the results, as previously described [37-40]. The results were graded in a semi-quantitative fashion as follows: (+) = 75%-100% of the neoplastic cells positive; (+/-) = 50%-75% of the neoplastic cells positive; (-/+) = 25%-50% of the neoplastic cells positive; rare = 10%-25% of the neoplastic cells positive; (-) = all neoplastic cells negative. The intensity of the staining and the pattern of positivity (nuclear, cytoplasmic diffuse, dot-like, granular, membrane-bound, etc.) were also recorded.

**EBV in situ hybridization**

In situ hybridization (ISH) was performed with fluorescein-labeled and antisense EBER 1&2 probes [41]. To detect the reaction, an alkaline phosphatase-conjugated monoclonal antibody specific to fluorescein was employed (provided by Dako, Denmark), followed by the New-Fuchsin substrate. Sections from an undifferentiated carcinoma of the nasopharynx represented the positive control for each run.

**Detection of bcl-2/IgH rearrangement**

A two step amplification of bcl-2/IgH rearrangement at the major (MBR) and minor (mcr) breakpoint regions was performed, according to previously described procedures [42]. In brief, PCR reactions were performed in a 50 μl final volume including 5 μl of DNA (extracted from paraffin-embedded tissue by QIAamp tissue kit, QIAGEN) or 2 μl of the first round PCR product. 50 mmol/l potassium chloride, 10 mmol/l TRIS hydrochloride, 2 mmol/l magnesium chloride, 200 μmol/l dNTPs, 20 pmol of each primer, and 2 U of Taq polymerase. The PCR conditions were as follows: denaturation at 94° for 30'; annealing at 56° (MBR) or 58° (mcr) for 30', extension at 72° for 30' (30 cycles), and final extension at 72° for 7' PCR amplification of the non rearranged bcl-2 gene, at the same conditions as above, was used as a control to confirm that amplifiable DNA had been extracted. Second round PCR samples (10 μl) were analyzed in a 2% agarose gel.

**Definition of response**

Complete remission (CR) was defined as disappearance of all detectable signs lasting longer than three months. Partial remission (PR) was defined as a reduction of at least 50% of the measurable tumor. No response (NR) was defined as reduction of less than 50% of the measurable tumor, or progression of the disease, or transient disease regression lasting less than one month.

**Results**

**Morphology**

All cases were characterized by a diffuse proliferation of large cells (mean diameter about 20 μm), which in seven instances were provided with variably shaped nuclei (round, oval, multilobated), dispersed chromatin, one or more medium-sized evident nucleoli, and a wide rim of clear cytoplasm (Figures 1–3). The remaining two cases revealed a bit more regular nuclear profile and a

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**Table 1. Panel of antibodies employed for the study.**

<table>
<thead>
<tr>
<th>Antibody (clone)</th>
<th>Reactivity</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD7/26+2B11</td>
<td>CD45</td>
<td>Dako, Denmark</td>
</tr>
<tr>
<td>L26</td>
<td>CD20</td>
<td>Dako, Denmark</td>
</tr>
<tr>
<td>IF8</td>
<td>CD21</td>
<td>Dako, Denmark</td>
</tr>
<tr>
<td>JCB117</td>
<td>CD79a</td>
<td>Prof. D Y. Mason</td>
</tr>
<tr>
<td>Polyclonal anti-CD3</td>
<td>CD3</td>
<td>Dako, Denmark</td>
</tr>
<tr>
<td>S6C6</td>
<td>CD10</td>
<td>Novocastra, UK</td>
</tr>
<tr>
<td>Polyclonal anti-x light chain</td>
<td>Ig kappa light chain</td>
<td>Dako, Denmark</td>
</tr>
<tr>
<td>Polyclonal anti-λ light chain</td>
<td>Ig lambda light chain</td>
<td>Dako, Denmark</td>
</tr>
<tr>
<td>124</td>
<td>Bcl-2 product</td>
<td>Dako, Denmark</td>
</tr>
<tr>
<td>PG-B6p</td>
<td>Bcl-6 product</td>
<td>Prof. B. Falini</td>
</tr>
<tr>
<td>Ber-H2</td>
<td>CD30</td>
<td>Dako, Denmark</td>
</tr>
<tr>
<td>CD1D1</td>
<td>CD15</td>
<td>Dako, Denmark</td>
</tr>
<tr>
<td>PG-M1</td>
<td>CD68</td>
<td>Prof. B. Falini</td>
</tr>
<tr>
<td>DO-7</td>
<td>p53</td>
<td>Dako, Denmark</td>
</tr>
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<td>DK22</td>
<td>HLA-DR</td>
<td>Dako, Denmark</td>
</tr>
<tr>
<td>E29</td>
<td>EMA</td>
<td>Dako, Denmark</td>
</tr>
<tr>
<td>MNN116</td>
<td>Cytokeratins</td>
<td>Dako, Denmark</td>
</tr>
<tr>
<td>β-hCG</td>
<td>β-hCG</td>
<td>Dako, Denmark</td>
</tr>
<tr>
<td>PLAP</td>
<td>PLAP 8B6</td>
<td>Dako, Denmark</td>
</tr>
<tr>
<td>Mb-1</td>
<td>Proliferation-associated nuclear antigen Ki-67</td>
<td>Dako, Denmark</td>
</tr>
</tbody>
</table>

Abbreviations: CD – cluster of differentiation, EMA – epithelial membrane antigen, hCG – human chorionic gonadotropin, PLAP – placental alkaline phosphatase

**Primary mediastinal large B-cell lymphomas for comparison**

Twenty-three typical examples of PMLBCL, which had been included in previous studies [22, 33], were retrieved from the files of the Unit of Pathology and Hematopathology of Bologna University, aiming to compare their morphologic and phenotypic features with those of the PRLBCLs of the present series. Histologic preparations were reviewed and phenotypic analysis (originally based on the determination of CD3, CD15, CD20, CD30, CD45, CD68, CD79a, and Ki-67) was extended to CD10, bcl-2 and bcl-6, i.e. molecules used for PRLBCL evaluation and object of recent reports in the literature [43-48]. The sections employed for immunohistochemistry were obtained from routinely processed tissue blocks [35, 36] and tested as above [37-40].
Figure 1. Neoplastic cells show a diffuse growth pattern and evoke a delicate fibrotic reaction (case number 1 of Table 2; hematoxylin & eosin, x150). Figure 2. At higher magnification, the lymphomatous elements display large size, pleomorphic nuclei, and a wide rim of clear cytoplasm; a giant cell is arrowed (case number 1 of Table 2; x600). Figure 3. Cell cytology at Giemsa staining in one of the tumors with clear cytoplasm (case number 9 of Table 2; x500). Figure 4. Hematoxylin & eosin stained preparation in one of the two cases with more regular nuclei and acidophilic cytoplasm; a giant cell is arrowed (case number 4 of Table 2; x250). Figure 5. Giemsa staining in the other case (case number 6 of Table 2; x600). Figure 6. Compartmentalization of lymphomatous cells as shown by Gomori silver impregnation (case number 1 of Table 2; x100). Figure 7. Expression of the bcl-2 gene product by the neoplastic cells (case number 3 of Table 2; x250). Figure 8. Positivity for the bcl-6 protein at the nuclear level (case number 5 of Table 2; x250).

large rim of cytoplasm, which appeared acidophilic on H.E. and grayish on Giemsa staining (Figures 4-5). No correlation was observed between cell morphology and inclusion in one of the subgroups defined by immunophenotyping and molecular analysis (Table 2). In all instances, some multinucleated giant cells – at times with
Reed–Sternberg-like features — were detected (Figures 2 and 4). Mitotic figures were numerous. Apoptotic bodies were also seen. Foci of necrosis with varying extension were encountered. Some small lymphocytes were dispersed through the neoplastic population or clustered tightly around capillaries. Reactive histiocytes were evenly distributed throughout the tumor. Sclerosis was always present in the form of broad collagen bands with focal hyalinization. In six cases, a compartmentalizing fibrosis was also observed, with interanastomosing collagenous bands (from 5 to 50 μm in width), which surrounded nests and cords of neoplastic cells (Figure 6). PAS stains accentuated the sclerosis but did not reveal glycogen in the lymphomatous elements. No features of pre-existing lymph node structure were found in any of the cases studied.

Immunohistochemistry and ISH

The basic phenotypic profile observed in each individual case is shown in Table 2. As expected, neoplastic cells turned out positive for expression of CD45 and B-cell markers (CD20 and CD79a), while negative in the search for CD3, CD15, CD68, EMA, cytokeratin, and onco-fetal antigens. The determination of cytoplasmic Ig light chains gave negative results in the seven cases with clear cells and showed a small amount of monocytic stained elements in the two cases with acidophilic cytoplasm. The expression of the bcl-2 and bcl-6 gene products was detected in six and five cases respectively (Figures 7 and 8). In five cases the latter two molecules were simultaneously expressed: one of these cases also showed positivity for CD10, while three were stained by the anti-HLA-DR specific antibody and three revealed a delicate network of CD21-positive follicular dendritic cells (FDCs). The only bcl-2+/bcl-6− tumor expressed CD30 and was positive for EBV at ISH. CD30 staining was found in two additional cases, while p53 expression was detected in 25%–50% of the neoplastic cells in three instances. The number of tumoral elements carrying the Ki-67 proliferation-associated nuclear antigen (as revealed by the Mib-1 monoclonal antibody) was on whole high (mean value: 74%, range: 40%–85%).

Molecular biology

Molecular analysis could only be performed in seven cases, since in the remaining two cases (both corresponding to the Menghini needle biopsies), inadequate material was available. Three of the analyzed cases showed rearrangement of the bcl-2 gene at MBR, thus suggesting the presence of the t(14;18) translocation (Table 2) (Figure 9). These three cases expressed HLA-DR and the bcl-2 and bcl-6 gene products, and showed variable expression of the CD10, CD30 and p53 molecules (Table 2).

![Image](image_url)
Table 3. Summary of clinical findings.

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age</th>
<th>Stage</th>
<th>B symptoms</th>
<th>Bulky disease</th>
<th>Other sites involved</th>
<th>Therapy (first line)</th>
<th>Result</th>
<th>Relapse</th>
<th>Therapy (second line)</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>1*</td>
<td>M</td>
<td>31</td>
<td>II</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>ProMace-CytaBom</td>
<td>CR</td>
<td>-</td>
<td>MAGRATH + ABMT</td>
<td>DOD/12 mos.</td>
</tr>
<tr>
<td>2*</td>
<td>M</td>
<td>73</td>
<td>IV</td>
<td>-</td>
<td>+</td>
<td>Kidney</td>
<td>RT</td>
<td>NR</td>
<td>-</td>
<td>DOD/8 mos.</td>
<td></td>
</tr>
<tr>
<td>3*</td>
<td>F</td>
<td>56</td>
<td>IV</td>
<td>-</td>
<td>+</td>
<td>Bone-marrow (FL/1) &amp; latero-cervical nodes</td>
<td>MACOP-B + RT</td>
<td>CR</td>
<td>-</td>
<td>CR/30 mos.</td>
<td></td>
</tr>
<tr>
<td>4*</td>
<td>M</td>
<td>62</td>
<td>II</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>CVP + Fluda + EDX</td>
<td>NR</td>
<td>-</td>
<td>MACOP-B</td>
<td>CR/30 mos.</td>
</tr>
<tr>
<td>5*</td>
<td>F</td>
<td>59</td>
<td>IV</td>
<td>-</td>
<td>+</td>
<td>Spleen, liver &amp; intestine</td>
<td>MACOP-B</td>
<td>PR</td>
<td>-</td>
<td>LEV</td>
<td>CR/71 mos.</td>
</tr>
<tr>
<td>6*</td>
<td>F</td>
<td>75</td>
<td>III</td>
<td>+</td>
<td>+</td>
<td>Bilateral axillary nodes</td>
<td>MICEP</td>
<td>NR</td>
<td>-</td>
<td>Leukeran</td>
<td>DOD/18 mos.</td>
</tr>
<tr>
<td>8*</td>
<td>M</td>
<td>34</td>
<td>II</td>
<td>-</td>
<td>+</td>
<td>Intestine</td>
<td>MACOP-B</td>
<td>CR</td>
<td>Lung &amp; kidney</td>
<td>DHAP + F-MACHOP + ABMT</td>
<td>DOD/23 mos.</td>
</tr>
<tr>
<td>9*</td>
<td>F</td>
<td>81</td>
<td>IV</td>
<td>-</td>
<td>+</td>
<td>Kidney</td>
<td>EDX</td>
<td>NR</td>
<td>-</td>
<td>-</td>
<td>DOD/3 mos.</td>
</tr>
</tbody>
</table>

Abbreviations: M – male; F – female; EDX – endoxan; Fluda – fludarabine; RT – radiotherapy on the abdomen; ABMT – autologous bone-marrow transplantation; NR – no response; PR – partial remission; CR – complete remission; DOD – died of disease; AWD – alive with disease; mos. – months; FL/I – follicular lymphoma grade I.

* Cases of putative follicle center cell derivation.

b Cases with borderline characteristics, finally included among putative follicular lymphomas.

c Cases without apparent normal counterpart.

**Revised diagnoses**

Based on the above-mentioned findings, cases 1–5 of Tables 2 and 3 were regarded as PRLBCLs of putative FCC derivation. The remaining four cases were classified as 'not otherwise specified' (NOS), since phenotypic and molecular analyses did not allow the definite recognition of a normal cell counterpart.

**Clinical findings**

The clinical features of the nine cases are summarized in Table 3. As reported in the material and method section, all patients primarily presented with a retroperitoneal tumor, which was sampled either during laparotomy or by ACT-guided needle biopsy. Following the diagnosis, two of them (cases 3 and 6 of Table 3) developed superficial lymphadenopathies at the cervical and axillary level, respectively: no additional biopsies were performed. Patients 1–5, who had the diagnosis of FCC-derived PRLBCL, were three males and two females (mean and median age: 56.2 and 52 years, respectively). Further sites of neoplastic involvement were recorded in three cases: these included the spleen, liver, intestine, kidney, bone marrow, and latero-cervical nodes. Interestingly enough, patient 3 of Table 2 and 3 showing bcl-2 gene rearrangement and latero-cervical lymphadenopathy, had simultaneous grade I FCC lymphoma [5] in the bone-marrow. Three subjects were in stage IV and two in stage II. Bulky tumor was detected in three instances. Systemic symptoms were not reported. At the time of this writing, three out of five patients are in CR, while the remaining two died of resistant or relapsing disease within 12 months. Patients 6–9 – with PRLBCL, NOS – were three females and one male (mean and median age: 62.2 and 57.5 years, respectively). Besides the retroperitoneal mass (bulky in three instances), they presented additional sites of lymphomatous involvement, including the lung, intestine, kidney, and axillary nodes. Systemic symptoms were recorded in two cases. Two subjects were staged IV, one III, and one II. All patients had an unfavorable clinical course and died of their disease in 3–23 months. Three of them experienced progressive tumor unresponsive to the therapies employed, while the remaining one achieved CR following the first line of therapy, but relapsed 12 months later in the lung and kidney, resulting refractory to all the approaches applied.

**Primary mediastinal large B-cell lymphomas for comparison**

The main clinical and pathologic findings are summarized in Table 4. At morphologic examination, 15 out of 23 examples of PMLBCL were characterized by a diffuse proliferation of polymorphous large cells with clear cytoplasm, while the remaining ones consisted of large elements with a lower degree of nuclear pleomorphism and a wide rim of acidophilic cytoplasm. Giant cells – at times with Reed–Sternberg morphology – were
variably observed. Mitotic figures were numerous. Foci of necrosis were often detected. Phenomena of sclerosis were usually found, which produced overt compartmentalization in 18 instances.

Upon immunohistochemical analysis, all cases appeared CD20+, CD79a+, CD45+, CD3−, CD15−, CD68−, and EMA−, with a mean Ki-67 marking of about 70%. CD30 was expressed in 19 instances (13 cases+, six cases−/+), but absent in the remaining four. The search for the Bcl-2 protein produced similar figures. Positivity for the Bcl-6 gene product was appreciated in 13 cases (8+, 1−/+; 4−/−), ten being negative. Only 4 out of 23 cases showed staining at the determination of CD10. Finally, HLA-DR expression was present in 16 samples, while lacking in the remainder.

**Discussion**

Malignant lymphomas primarily arising in the retroperitoneum have rarely been the object of detailed clinicopathologic reports [1–4]. They were considered to be mostly of FCC derivation and associated with sclerosis [2, 4]. When effective chemotherapy was not available, the latter finding was regarded as a favorable prognostic indicator [2]. The nine cases of the present series expand the spectrum of our knowledge on PRLBCL by showing its heterogeneity in terms of morphology, phenotype, molecular characteristics, and outcome.

Although the comparison between our and previous studies is indeed difficult because of the different approaches employed, some findings merit a brief comment. The age and sex of the patients of our series are in line with the data of the literature on retroperitoneal lymphomas [4]. Conversely, to Waldron et al. [4], however, we did not observe residual nodal structure nor hints of follicular growth pattern. Although this might be due to inadequate sampling in our material, the seven biopsies obtained during laparotomy measured at least 5 cm² and were multiple in three instances. Thus, in light of the present tendency to avoid massive surgery and to apply minimally invasive procedures for the diagnosis of deep masses, morphology may be inadequate to assess whether a PRLBCL represents the transformed phase of a less aggressive FCC lymphoma or not. In the present series, such a transformation – which seems to be of prognostic value [45, 53] – could be suspected at pure microscopic examination in only one patient due to the detection of an indolent follicular lymphoma in the bone-marrow. Our data also contrast with old reports as to the postulated positive role of sclerosis [2]; in fact, in spite of the constant presence of evident fibrosis, six of our patients died of their disease. On the whole, conventional light microscopy in our series did not provide useful elements to achieve a more accurate classification and prognostic prediction. The different cytologic composition – clear cells vs. acidophilic cells – possibly reflected the presence of diverse amounts of organelles in the cytoplasm of the neoplastic elements, as suggested by the lack of detectable Ig in the former and the occurrence of occasional elements bearing monotypic Ig in the latter.

By contrast, phenotypic analysis and molecular biology considerably contributed to the better understanding of our cases.

Five tumors were regarded as consistent with FCC derivation [5–7, 44, 48] (cases 1–5 of Tables 2 and 3). At immunohistochemistry, they all expressed the bcl-2 and bcl-6 products, three were HLA-DR-positive, three contained CD21+ FDCs, and one stained for CD10. On molecular grounds, bcl-2 gene rearrangement was detected in three instances. In particular, the bcl-6 protein seemed to represent an excellent indicator of FCC derivation in this series, more effective than CD10, which at times can be lost during transformation [48]. The bcl-6 gene product is a zinc finger transcription factor regularly expressed by B-cells passing through germinal centers, which occurs independently of a 3q27 chromosomal aberration [46–48, 52, 53]. A matter of speculation might be whether our cases represented de novo LBCLs or transformed FCC lymphomas. P53 overexpression – which usually reflects mutations of exons five to eight of the corresponding gene – might to some extent support the latter hypothesis, since it has been claimed to play a role in tumor transformation [49–51]. Clinically, the five patients with PRLBCL of putative FCC derivation showed variable response to therapy: two achieved and still maintain CR, one is alive in PR, and two died of disease (Table 3). The remaining four cases were classified as diffuse LBCL/NOS (numbers 6–9 of Table 2 and 3). Of these, three (numbers 6–8) were negative at the determination of bcl-2, bcl-6, CD10, p53, and HLA-DR, lacked FDCs, and – in the two instances with enough material available – showed no rearrangements of the bcl-2 gene. The fourth case (number 9) did not carry bcl-6, HLA-DR, FDC, and bcl-2 rearrangement, but displayed EBV
genomic integration. The latter finding might explain the positivities for bcl-2 and CD30 observed in this case [54]; however, it might also have also contributed to bcl-6 down-regulation [55], thus making tumor subclassification difficult. The four patients with PRLBCL/ NOS experienced a very aggressive clinical course with involvement of the lung, kidney and intestine and died of their disease in spite of the effective therapies employed (Table 3).

As a result of some similarities between PRLBCL and PMLBCL (e.g. cytology, tendency to extranodal spread, etc.), we decided to compare the clinical, morphologic and phenotypic features of the present series with those of 23 typical PMLBCLs, which were included in previous reports [22, 33]. In particular, no significant differences were observed at light microscopy between the two processes, since the examples of PMLBCL were also characterized by clear or acidophilic cells and occurrence of sclerosis with frequent compartmentalization. At immunophenotypic analysis, PMLBCLs showed a higher frequency of CD30 positivity and a higher ratio of CD30+ cells in the positive cases. On the other hand, they appeared very heterogeneous when tested with the antibodies against bcl-2, bcl-6, and CD10. Interestingly enough, positivity for the bcl-6 gene product was detected in 13 out of 23 PMLBCLs examined, although bcl-6 gene mutations have rarely been found in this tumor [23, 53]. Our findings suggest that the expression of bcl-6 and CD10 in PMLBCL merits further attention, also in light of the fact that – to the best of our knowledge – no extensive reports on the topic exist. A further point to be assessed in future studies, is MAL protein expression in PRLBCL. This protein – which is characteristically detected in PMLBCL [34] – was investigated in our series; the staining was reliable in only two cases, as shown by the internal controls: it turned out to be negative in both (data not shown). Clinically, PRLBCL differed from PMLBCL in terms of median age, male/ female ratio, incidence of stage I–II, and CR percentage (Table 4). On the whole, our findings do not allow speculation as to the existence of a possible histogenetic link between PMLBCL and PRLBCL. However, they strengthen the concept, already reported in the validation study of the R.E.A.L. Classification [56], that the knowledge of the primary site of the process is valuable in diagnosing these peculiar tumors.

Finally, one should always keep in mind that in biopsies actually involved with PRLBCL, the presence of sclerosis and compartmentalization, along with cytological details may suggest a misdiagnosis of germ cell tumor, undifferentiated carcinoma or even syncitial Hodgkin’s disease; i.e. processes that need different therapeutic strategies. Immunohistochemistry allows, however, easy differentiation from these neoplasms by showing in PRLBCL expression of CD45 and B-cell markers and negativity for CD15, EMA, cytokeratin, and onco-fetal antigens [38].

In line with previous reports on the broader group of DLBCLs [5–7, 48, 52], the present study shows the molecular and clinical heterogeneity of PRLBCLs, thus strengthening the need of employing tools other than morphology for their possible subclassification, including the most recently developed molecular techniques [57]. Because of the limited number of cases enrolled, our study cannot not represent the final answer on this topic, but aims to stimulate concerted international scientific cooperation in order to collect a larger series of patients.

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