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Malignant Lymphomas

The pathologist's view point. Part II – aggressive lymphomas

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ABSTRACT

Background and Objectives. **The REAL/WHO classification constitutes a new tool for the better understanding and treatment of malignant lymphomas. The authors focus on the key features of aggressive B- and T-cell lymphomas, aiming to contribute to the cross-talk between pathologists and clinicians.**

Data sources and methods. **Each lymphoma entity is analyzed on the basis of the most representative contributions in the literature and the authors' experience gained in studying more than 20,000 lymphoid tumors over a 20-year period.**

Results. **Guidelines for diagnosis and areas of interest for future clinico-pathologic studies are identified and discussed. Within this context, selected data obtained by the application of novel markers are presented.**

Interpretation and Conclusions. **The present knowledge and organization of malignant lymphomas now make the development of tailored therapies a feasible goal.**

Key words: aggressive lymphoma, morphology, phenotype, genotype, behavior

In the clinical setting, the term *aggressive lymphoma* has recently substituted *high grade malignant lymphoma*, previously quoted in both the *Working Formulation* (WF)[1] and *Updated Kiel Classification* (UKC):[2,3] it is currently applied to lymphoid tumors characterized by survival measurable in weeks or months if not treated.[5,6] This term was not adopted by the *Revised European-American Lymphoma* (REAL) classification[4] for the reasons already reported in Part I (see *Clinical categorization of malignant lymphomas* paragraph). Aggressive lymphomas may show nodal, extranodal, systemic, or leukemic presentation, more often associated with rapidly growing masses and systemic symptoms. The main histologic varieties of aggressive lymphoma are listed in Table 1: they include neoplasms which are derived either from precursors or peripheral elements and can indifferently consist of small or large cells.

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B- and T-cell precursor (lymphoblastic) lymphoma/leukemia

There is no chance of distinguishing between B- and T-cell lymphoblastic lymphomas/leukemias (LbL/Ls) by morphologic criteria alone.[4] In fact, they both consist of small-medium sized elements, which display a high nuclear/cytoplasmic ratio, frequent irregular nuclear profile, condensed chromatin, and inconspicuous nucleoli (Figures 1a and 1b). The tumoral growth is characterized by frequent mitotic figures and apoptotic bodies, which attract numerous macrophages (Figures 1a and 1b). The distinction between the two forms does, however, become feasible on phenotypic grounds: this distinction is of paramount importance, since B- and T-cell tumors require different therapeutic approaches.[7-9] In particular, immunohistochemistry, which can now be easily performed in routine sections thanks to the availability of new antigen retrieval techniques,[10] allows the subclassification of LbL/Ls into subtypes (pro-B, pre-B, B-mature, pre-T, from cortical thymocytes, and from medullary thymocytes) according to the combination of a series of key-markers (i.e. CD34, TdT, BSAP, CD79a, CD20, CD10, CIgμ, SIg, CD1a, CD2, CD3, CD4, CD5, and CD8)[11] (Figures 1c-f).

The detection of cytogenetic and/or molecular aberrations is prognostically relevant.[4,12-19] Among tumors derived from B-cell precursors, t(12;21) with the formation of the TEL-AML1 fusion gene, t(3;21) and a hyperdiploid pattern between 51 and 65 represent favorable indicators; t(9;22), t(14;19), and a hypodiploid pat-

Table 1. Aggressive lymphomas.

B-cell neoplasms

Precursor B-cell neoplasms
- Precursor B-lymphoblastic leukemia/lymphoma

Peripheral B-cell neoplasms
- Mantle cell lymphoma
- Follicular lymphoma grade III
- Diffuse large B-cell lymphoma
- Burkitt's lymphoma/Burkitt cell leukemia

T-cell and putative NK-cell neoplasms

Precursor T-cell neoplasms
- Precursor T-lymphoblastic lymphoma/leukemia

Peripheral T- and NK-cell neoplasms
- T-cell prolymphocytic leukemia
- Aggressive NK-cell leukemia
- Adult T-cell lymphoma/leukemia (HTLV-1+)
- Extranodal NK/T-cell lymphoma, nasal type
- Enteropathy-type T-cell lymphoma
- Hepatosplenic T-cell lymphoma
- Subcutaneous panniculitis-like T-cell lymphoma
- Peripheral T-cell lymphomas not otherwise specified (NOS)
- Angioimmunoblastic T-cell lymphoma
- Anaplastic large cell lymphoma, primary, systemic

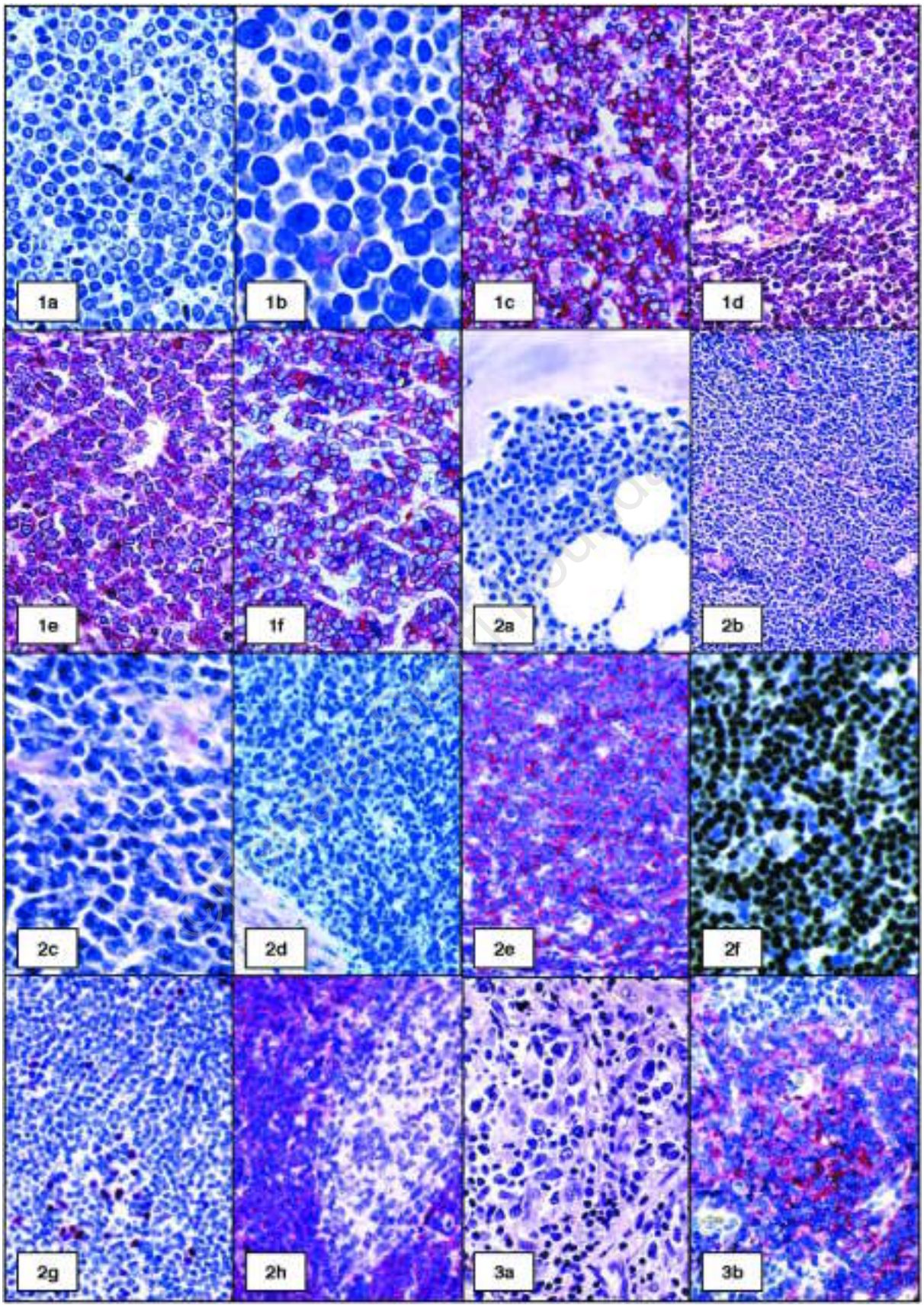
tern herald a poor outcome; 6q-, 9p-, 12p-, and a triploid, tetraploid or hyperdiploid (below 51) pattern have an intermediate prognostic value. About 30% of T-lymphoblastic lymphomas show translocations, which on the one hand involve α/δ or β/γ T-cell receptor (TCR) genes (at bands 14q11 and 7q34, respectively) and on the other partners encoding for transcription factors (e.g. MYC, TAL1, RBTN1, RBTN2, and HOX11). In 25% of the cases, instead of a translocation there is a microscopic deletion in the regulatory region of the TAL1 gene at 5'. Furthermore, about 30% of T-lymphoblastic leukemias display a deletion in 9p with loss of the oncosuppressor gene $p16^{ink4a}$. Finally, in infants with neoplasms from lymphoid precursors, irrespectively of the B- or T-cell lineage, abnormalities involving 11q23 and the MLL gene indicate a poor prognosis: thus, rapid and efficient detection of MLL rearrangements is relevant in determining therapeutic strategy.[18]

On clinical grounds, B-lymphoblastic tumors more often show leukemic manifestations, while the T-cell ones are characterized by a rapidly growing mediastinal mass. The tendency of these tumors to involve the central nervous system (CNS) and gonads requires CNS prophylaxis at presentation and restaging at the time of therapy discontinuation, respectively.[4,12]

Mantle cell lymphoma

This tumor was erroneously regarded as a low-grade malignant process by both the WF[1] and UKC,[2,3] that termed it small-cleaved cell lymphoma and centrocytic lymphoma, respectively. At the state of the art, mantle cell lymphoma (MCL) belongs to the body of aggressive lymphomas, since its median survival is below 3 years.[4,20,21] It involves the bone marrow in 70% of cases (Figure 2a) (with characteristic paratrabecular diffusion), the intestine in 30% of cases (where it can also present primarily), and the peripheral blood in 20-25% of cases (producing a leukemic picture).[4,26] Conventional regimens are not effective in the treatment of MCL: recent data on a limited number of cases suggest a good response to high dose therapies followed by autologous bone marrow transplantation.[21] At conventional light microscopy,[4,20] the tumor can show a mantle zone, nodular or diffuse growth pattern (Figure 2b). In the first two instances, the differential diagnosis with a marginal zone (MZL) or a follicular center lymphoma (FCL) can be difficult. Tumors with a diffuse growth pattern are more easily recognized, although the distinction from B-cell chronic lymphocytic leukemia (B-CLL), lymphoplasmacytic lymphoma (LPL), MZL, and FCL may at times be problematic. The commonest form of MCL consists of small-medium sized elements, showing a narrow rim of slightly basophilic cytoplasm and indented nuclei, with moderately dispersed chromatin and a small central nucleolus (Figure 2c). In this context, perivascular deposits of hyaline material and acidophilic histiocytes are often encountered. More rarely, MCL is characterized by the occurrence of small round cells, blastoid elements or a polymorphic population, which can lead to a misdiagnosis of B-CLL, LbL/L or diffuse large B-cell lymphoma (DLB-CL), respectively (Figure 2d).

Immunohistochemistry is of paramount importance for the identification of MCL: in fact – besides the positivities for CD19, CD20, CD22, and CD79a – it shows expression of CD5 and cyclin D1[4,20] (Figures 2e and 2f). The latter indicates bcl-1 gene rearrangement, which is due to the occurrence of t(11;14) in 70% of cases.[4,20] It should be underlined that bcl-1 gene rearrangement is characteristic, but not patho-gnomonic of MCL, since it can occasionally occur also in other lymphoid tumors.[22] In all instances, there is occasional positivity for IRF4 and regular expression of BSAP (23) and bcl-2 gene product (Figures 2g and 2h): although the latter does not depend on the presence of t(14;18), nevertheless it produces protection of neoplastic cells from apoptosis.[4,20] The search for CD10, CD23, CD68, CD72, and bcl-6 product produces constantly negative results.[4,20,24,25] The above mentioned phenotypic profile allows easy distinction of MCL from B-CLL ($CD5^+$, $CD23^+$, $IRF4^{+/-}$,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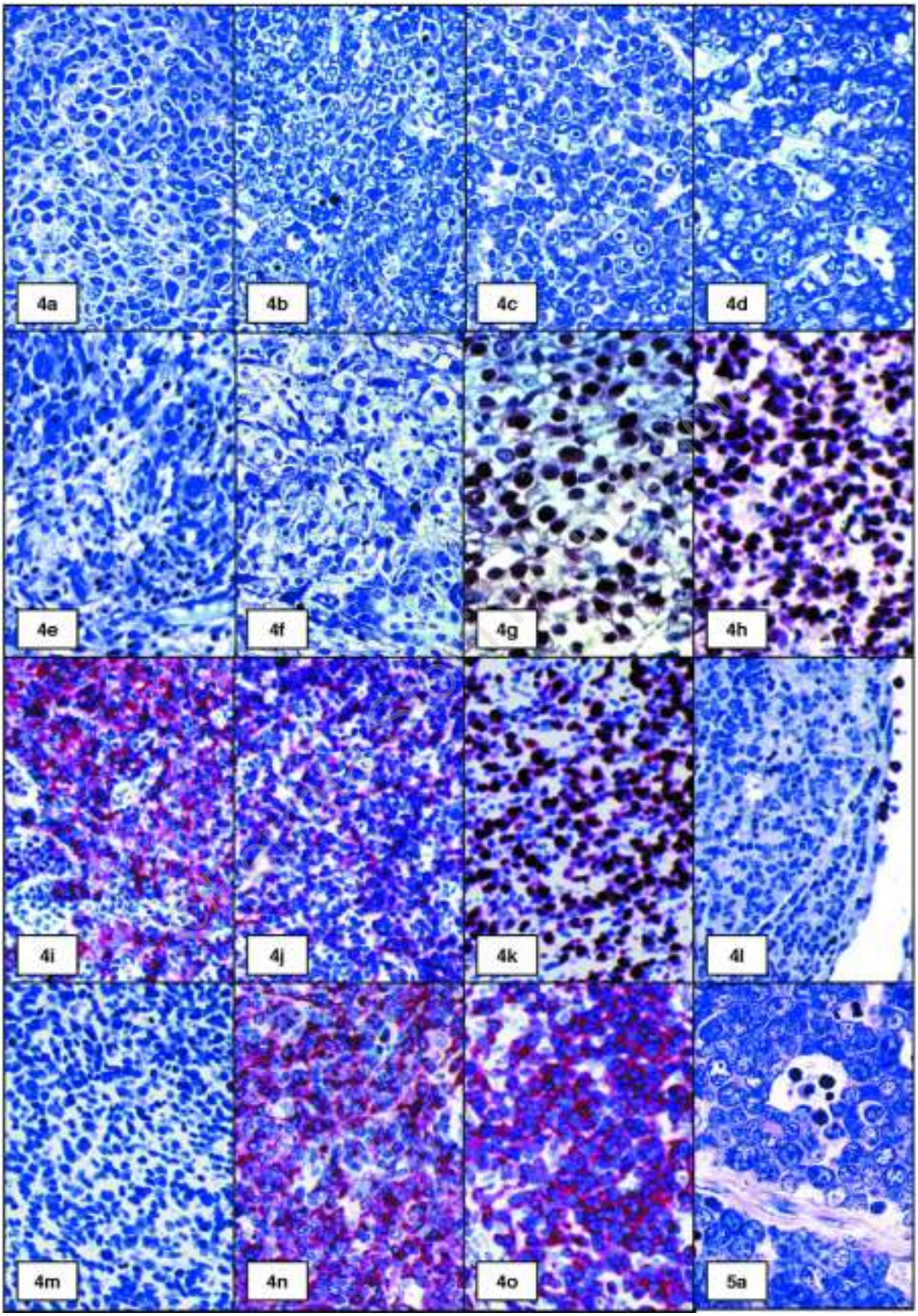
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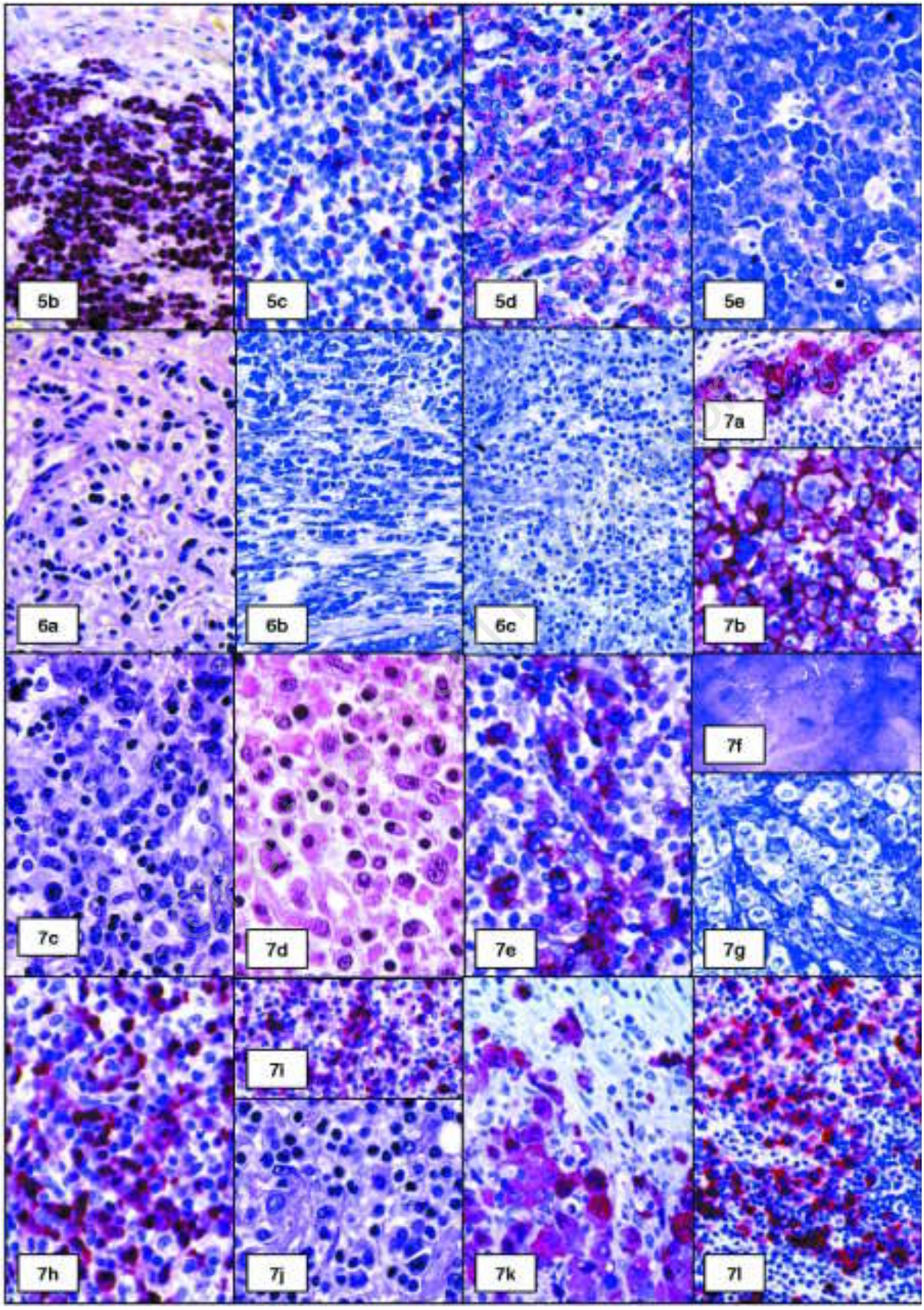
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Legends to figures.

Figure 1. (page 1310). Precursor (lymphoblastic) lymphoma / leukemia: a) cell morphology allows the recognition of the lymphoblastic nature of the tumor, but does not discriminate between B- and T-cell forms (Giemsa; ×400); b) neoplastic cells show size variability, high nuclear / cytoplasmic ratio, condensed chromatin, and frequent mitotic figures (Giemsa; ×600); c) CD34 positivity in a pro-B form (immunoalkaline phosphatase technique in paraffin sections; ×300); d) TdT staining in a pro-B form (immunoalkaline phosphatase technique in paraffin sections; ×300); e) CD79a expression in a pro-B form (immunoalkaline phosphatase technique in paraffin sections; ×300); f) CD3 positivity in a T-lymphoblastic lymphoma (immunoalkaline phosphatase technique in paraffin sections; ×400).

Figure 2. (page 1310). Mantle cell lymphoma: a) paratrabecular infiltration of the bone marrow (Giemsa; ×300); b) diffuse effacement of the normal lymph node structure; note some perivascular deposits of hyaline material around vessels (Giemsa; ×100); c) cytological details of mantle cell lymphoma of the common type (Giemsa; x 500); d) mantle cell lymphoma of the polymorphic type: neoplastic cells show a certain variability of size and shape (Giemsa; ×300); e) CD5 expression by neoplastic cells (immunoalkaline phosphatase technique in paraffin sections; ×300); f) neoplastic cells overexpress cyclin D1 at the nuclear level (immunoperoxidase technique in paraffin sections; ×500); g) IRF4 is detected in a few neoplastic cells (immunoalkaline phosphatase technique in paraffin sections; ×300); h) bcl-2 staining shows positivity of neoplastic cells and negativity of a residual germinal center (immunoalkaline phosphatase technique in paraffin sections; ×300).

Figure 3. (page 1310). Follicle center lymphoma grade III a) the tumor shows nodular aggregation and mainly consists of centroblasts (hematoxylin and eosin; ×400); b) expression of the CD10 molecule by neoplastic cells (immunoalkaline phosphatase technique in paraffin sections; ×300).

Figure 4. (page 1311). Diffuse large B-cell lymphoma: a) marked cellular pleomorphism of the neoplastic growth (Giemsa; ×400); b) centroblastic variant (Giemsa; ×400); c) immunoblastic variant (Giemsa; ×400); d) anaplastic variant (Giemsa; ×400); e) T-cell-rich B-cell lymphoma: neoplastic cells arrowed (Giemsa; ×400); f) primary mediastinal large B-cell lymphoma; note the clear cytoplasm of most neoplastic cells (Giemsa; ×400); g) pyothorax-associated lymphoma: integration of EBV in the genome of lymphomatous elements as shown by *in situ* hybridization with EBER1 / 2 probes (immunoalkaline phosphatase technique in paraffin sections; ×500); h) bcl-6 positivity in a diffuse large B-cell lymphoma (immunoalkaline phosphatase technique in paraffin sections; ×400); i) the same case expresses CD10 (immunoalkaline phosphatase technique in paraffin sections; ×300); j) and the bcl-2 gene product (immunoalkaline phosphatase technique in paraffin sections; ×300); k) bcl-6 positivity in another example of diffuse large B-cell lymphoma (immunoalkaline phosphatase technique in paraffin sections; ×300); l) the same case is negative at determination of CD10 (immunoalkaline phosphatase technique in paraffin sections; ×300); m) and the bcl-2 gene product (immunoalkaline phosphatase technique in paraffin sections; ×300); n) CD79 positivity in a diffuse large B-cell lymphoma with immunoblastic morphology (immunoalkaline phosphatase technique in paraffin sections; ×300); o) in the same case, expression of the VS38C molecule (immunoalkaline phosphatase technique in paraffin sections; ×400).

Figure 5. (pages 1311 and 1312). Burkitt's lymphoma: a) cytological composition; note the homogeneous cell size, chromatin and nucleoli distribution, narrow and deeply basophilic cytoplasm, tumor cell cohesiveness, and starry-sky pattern (Giemsa; ×600); b) blc-6 expression (immunoalkaline phosphatase technique in paraffin sections; ×400); c) negativity at determination of bcl-2 (immunoalkaline phosphatase technique in paraffin sections; ×400); d) all neoplastic cells express CD10 (immunoalkaline phosphatase technique in paraffin sections; ×400); e) Burkitt's-like morphology: neoplastic cells show a certain variability in size and shape (Giemsa; ×400).

Figure 6. (page 1312). Peripheral T-cell lymphoma, non-anaplastic: a) peripheral T-cell lymphoma, nasal-type: note the pleomorphism of the neoplastic population and the extensive necrosis (hematoxylin and eosin; ×600); b) peripheral T-cell lymphoma, intestinal-type: note the cell-size variability of the tumoral growth, as well as the presence of an eosinophilic component (Giemsa; ×400); c) peripheral T-cell lymphoma, the angioimmunoblastic type: note the rather loose cellularity and the prominent arborizing venules with high endothelium (Giemsa; ×400).

Figure 7. (page 1312). Anaplastic large cell lymphoma: a) intrasinusoidal diffusion of neoplastic cells (immunoalkaline phosphatase technique in paraffin sections; ×500); b) strong expression of CD30 molecule both at the membrane level and in the Golgi area (immunoalkaline phosphatase technique in paraffin sections; ×400); c) common-type variant: note the size of the cells, the kidney-shaped nuclei and the basophilia of the cytoplasm (hematoxylin and eosin; ×400); d) lympho-histiocytic variant: neoplastic cells are obscured by reactive macrophages with eccentric nuclei (hematoxylin and eosin; ×400); e) in the same case as in Figure 1d, CD30 immunostaining allows easy recognition of neoplastic cells (immunoalkaline phosphatase technique in paraffin sections; ×500); f) Hodgkin's-like variant: the tumor shows a nodular growth pattern and evokes collagen band deposition around the nodules (hematoxylin and eosin; ×25); g) at higher magnification, the tumor consists almost completely of large anaplastic cells (Giemsa; ×400); h) common-type tumor: ALK protein is expressed at both the nuclear and cytoplasmic levels (immunoalkaline phosphatase technique in paraffin sections; ×400); i) small-cell type: ALK protein is expressed at both the nuclear and cytoplasmic levels in the large cells; note the nuclear positivity of small-medium sized elements, which also belong to the neoplastic growth and carry t(2;5) (immunoalkaline phosphatase technique in paraffin sections; x 200); j) small-cell type: histologic features of the same case as shown in Figure 7i (hematoxylin and eosin; ×400); k) Hodgkin's-like type: ALK protein expression by neoplastic cells; the case is the same as in Figures 7f and 7g (immunoalkaline phosphatase technique in paraffin sections; ×500); l) ALK protein expression is limited to the cytoplasm: the tumor carries inv(2)(p23;q35) (immunoalkaline phosphatase technique in paraffin sections; ×300).

BSAP$^+$), LPL (CD5$^-$, CD10$^-$, CD68$^-$, CD72$^-$, bcl-6$^-$, IRF4$^+$, BSAP$^+$), MZL (CD5$^-$, CD10$^-$, CD68$^{+/-}$, CD72$^{-/+}$, bcl-6$^-$, IRF4$^{-/+}$, BSAP$^{-/+}$), and FCL (CD5$^-$, CD10$^+$, bcl-6$^+$, IRF4$^-$, BASP$^{+/-}$).[4,20,23-25] The antibodies anti-CD21, CD23, and CD35 show a loose meshwork of follicular dendritic cells, which originate from vessels with hyaline wall or represent remnants of pre-existing germinal centers, which are in turn CD10$^+$, bcl-6$^+$, and bcl-2$^-$ (Figure 2h). The Ki-67 marking varies from case to case: a multivariate analysis on 304 cases has recently shown that patients with higher proliferative indices have a more aggressive clinical course.[26]

Follicular lymphoma, grade III

This tumor, which shows either a follicular or follicular and diffuse growth pattern, contains more than 15 centroblasts/high power field[4] (Figure 3a). The blast count should be performed by an experienced pathologist, because of the risk of including follicular dendritic cells and histiocytes among neoplastic elements. This might lead to overestimation of the frequency of grade III follicular lymphoma and over-treatment of a percentage of patients. Mitotic figures are numerous. Besides positivity for B-cell markers (BSAP, CD19, CD20, CD22, CD79a), immunohistochemistry displays expression of CD10 and bcl-6 molecules (Figure 3b) (Table 2).[27,28] The search for the bcl-2 gene product can at times provide negative results.[29,30] The latter finding should be taken into consideration for the differential diagnosis between FCL grade III and follicular hyperplasia: in fact, while bcl-2 positivity supports the diagnosis of malignant lymphoma, negativity does not exclude it.[29] Therefore, if a FCL is suspected at conventional light microscopy, but the bcl-2 staining is negative, other investigations should be performed, such as the determination of intracytoplasmic and/or surface Ig, the molecular analysis of Ig encoding

Table 2. Phenotypic profile of 110 aggressive B-cell lymphomas.

	CD3⁺	*CD20⁺*	*CD79a⁺*	*CD10⁺*	*Bcl-2⁺*	*Bcl-6⁺*	*CD30⁺*	*CD138⁺*	*IRF4⁺*
FCL/III	0/12	12/12	12/12	11/12	9/12	12/12	2/12	0/12	0/12
DLBC/NOS	0/35	35/35	35/35	17/35	27/35	24/35	11/35	0/10	16/20
DLBC/CB	0/5	5/5	5/5	3/5	3/5	5/5	0/5	0/5	1/3
DLBC/IB	0/10	8/10	10/10	0/10	8/10	0/10	2/10	10/10	5/5
DLBC/ANA	0/2	2/2	2/2	0/2	1/2	0/2	2/2	0/2	1/2
PMLBC	0/24	24/24	23/24	4/24	20/24	14/24	20/24	ND	ND
BT	0/15	15/15	15/15	15/15	0/15	15/15	0/15	0/15	ND
BA	0/7	7/7	7/7	0/7	7/7	0/7	0/7	0/7	ND

FCL/III: Follicle center cell lymphoma, grade III. DLBC/NOS: diffuse large B-cell lymphoma, not otherwise specified. DLBC/CB: diffuse large B-cell lymphoma, centroblastic. DLBC/IB: diffuse large B-cell lymphoma, immunoblastic. DLBC/ANA: diffuse large B-cell lymphoma, anaplastic. PMLBC: primary mediastinal large B-cell lymphoma. BT: Burkitt's lymphoma, typical. BA: Burkitt's lymphoma, atypical. +: expression of the molecule in question by neoplastic cells. ND: not done.

genes, and the bcl-2 gene status.[4] Recent studies from the Würzburg group reveal that FCL grade III can carry the same chromosomal aberrations as DLBCL (i.e. bcl-6 gene rearrangements, 1qinv, and 6qdel) (*Hans-Konrad Müller-Hermelink, personal communication, IX ILSG Meeting, Toulouse, October 11-13, 1999*): this observation strengthens the concept that DLBCL may derive from a pre-existing FCL.[31,32]

In keeping with its morphologic, kinetic and molecular characteristics, FCL grade III runs a more aggressive clinical course than the grade I and II forms.[6,33,34]

Diffuse large B-cell lymphoma

The tumor may develop *de novo* or derive from a previous indolent lymphoma, such as B-CLL, LPL, MZL, or FCL.[4]

On morphologic grounds,[4] DLBCL consists of large cells (mean diameter = 20 μm), more often characterized by pronounced nuclear pleomorphism, prominent nucleoli, and a rim of basophilic cytoplasm (Figure 4a). The neoplasm grows diffusely, at times spreading through residual sinuses. Mitotic figures are always numerous. Some macrophages phagocytizing nuclear debris can be encountered. In a minority of cases one cytotype predominates over the others, thus allowing the subclassification of the process into the following forms: centroblastic, immunoblastic, anaplastic, or with large multilobated nuclei (Figures 4b-d). A further morphologic variant of the tumor, characterized by a high content of reactive T-lymphocytes, is termed *T-cell rich B-cell lymphoma* (Figure 4e) and can be confused with lymphocyte-predominant Hodgkin's disease.[4,35-37] This variant occasionally shows prominent angiocentricity, thus producing the picture originally described as *lymphomatoid granulomatosis*.[38] There is no consensus on the usefulness of distinguishing histologic subtypes of DLBCL: some groups have reported a more aggressive clinical course in patients with immunoblastic tumor.[39,40]

DLBCLs are also distinguished into different subtypes according to disease presentation: primary mediastinal or thymic,[41-44] intravascular,[45,46] body cavity-based,[47,48] and pyothorax-associated.[49] The primary mediastinal form more often occurs in females in the fourth decade of life and presents in stage I or II with bulky tumor and superior vena cava syndrome (Figure 4f). This variety seems to be very sensitive to third generation chemotherapy regimens, such as the MACOP-B; at relapse, it characteristically involves the liver, CNS, kidney, intestine, and gonads. The intravascular variant usually occurs at extranodal sites, such as the skin, lung or brain. The so-called body cavity-based or primary effusion lymphoma does not stem from contiguous neoplastic masses, is observed in immunodeficient patients (mainly HIV^+), is associated with HHV8 infection, and displays a peculiar phenotypic profile (with regular expression of CD45, HLA-DR, EMA, CD30, CD38, and CD77, and lack of B- and T-cell markers). The derivation of this tumor from peripheral B-cells is supported by the observed clonal rearrangements of Ig encoding genes.[47] Finally, pyothorax-associated DLBCL develops in patients with a previous history of tubercular empyema, infiltrates the chest wall extensively, and shows regular EBV integration in the genome of neoplastic elements (Figure 4g).

On phenotypic grounds (Table 2), at least three main groups of DLBCL can be distinguished: $CD10^+/bcl\text{-}6^+$, $CD10^-/bcl\text{-}6^+$, $CD10^-/bcl\text{-}6^-$.[50,51] The first group corresponds to neoplasms which derive from germinal center cells – either *de novo* or following transformation of a pre-existing FCL – and are characterized by strong-moderate expression of CD20, CD79a, and bcl-2 protein (Figures 4h-j). The remaining two categories appear to be more heterogeneous. In fact, they include tumors with the following phenotypic profiles: a) $bcl\text{-}6^+/bcl\text{-}2^+/CD10^-$ (possibly also derived from germinal center cells), b) $bcl\text{-}6^+/bcl\text{-}2^-/CD10^-$ (with controversial histogenesis), c) $bcl\text{-}6^-/bcl\text{-}2/CD10^-/CD20^{-/+}/CD30^{-/+}/CD79a^+/CD138^+/EMA^{+/-}$/mum-$1^+$/$VS38c^+$/$CIg^+$ (with immunoblastic morphology), and d) $bcl\text{-}6^-/bcl\text{-}2^-/CD10^-/CD20^+/CD30^+/CD79a^+/CD138^-/EMA^{+/-}$/$VS38c^-$ (with anaplastic morphology) (Figures 4k-o). Interestingly, the leukocyte common antigen/CD45 is absent in about 30% of DLBCLs of the immunoblastic and anaplastic types.[4,52] The expression of the bcl-2 gene product deserves special attention: in fact, three

reports in the literature – based on large series of cases – suggest that DLBCLs carrying this protein run a more aggressive clinical course than the bcl-2 negative ones, as shown by the significant differences in terms of overall survival and disease-free survival.[53-55]

Numerous cytogenetic and molecular aberrations have been detected in DLBCLs: among these, some have a higher incidence, such as rearrangements of the bcl-2, bcl-6, and c-myc genes, +5, +6, +7, +18, 6qdel, and breakpoints at 1q2-23, 6q21-25, and 14q11-12.[53-58] These aberrations are not always associated with overexpression of the corresponding gene products.[53-55] Furthermore, their possible prognostic relevance, greatly emphasized in the early 1990s,[56] has recently been questioned, some authors claiming that they play a major role only in the process of lymphomagenesis.[54-58] However, very recent data obtained by DNA microarrays suggest that the diversity in gene expression among DLBCLs apparently reflects the variation in tumor proliferation rate, host response and differentiation state of the tumor. In particular, patients whose disease has a germinal-center B-cell-like molecular pattern might have a more favorable course than those with an activated B-cell-like profile.[59]

Burkitt's lymphoma

Burkitt gave the prototypic description of this tumor in 1955, based on series of cases observed in Central Africa.[60] The neoplasm appears monomorphic and consists of medium-sized elements, with deeply basophilic, vacuolated cytoplasm and round-oval nuclei, showing reticulated chromatin and 2-6 small nucleoli[4] (Figure 5a). The process is characterized by a cohesive growth pattern, extremely numerous mitotic figures, abundant apoptotic bodies, and frequent macrophages, which produce a *starry-sky* appearance[4] (Figure 5a).

At present, two types of Burkitt's lymphoma (endemic and sporadic), as well as a Burkitt's-like tumor are distinguished.[4]

Endemic Burkitt's lymphoma is characteristically observed in the malarial areas of Central Africa, where it affects children and young adolescents, involving the mandible or gonads and showing regular EBV integration in the genome of neoplastic cells. Its response to therapy is quite good. The sporadic form, which is definitely isomorphic, occurs in Western countries among young people and adults, the latter often being sero-positive. The tumor can present in the intestine, rectum, gonads or CNS, is highly aggressive and requires the administration of protocols normally applied to patients with acute Lb leukemias.[19] Only 25% of HIV-negative individuals display EBV integration in the genome of neoplastic cells, while more than a half of the HIV-positive ones show such an association.[61]

At phenotypic analysis (Table 2), both endemic and sporadic Burkitt's lymphomas express CD19, CD20, CD22, CD79a, CD10, and bcl-6 protein, while they regularly lack the bcl-2 gene product[62] (Figures 5b and 5d). Furthermore, they systematically carry a series of translocations [t(8;14), t(2;8), t(8;22)], which involve the c-myc gene and cause overexpression of its product with resulting entrance of all neoplastic elements in the cell cycle.[4] While endemic Burkitt's lymphoma is generally associated with t(8;14), the sporadic form can indifferently show one of the three above mentioned aberrations.[4]

Burkitt's-like lymphoma represents a highly controversial issue. It was originally quoted as a provisional entity in the REAL Classification.[4] In 1997, at the Consensus Conference held at Airlie House in Virginia (USA), it was matter of discussion among clinicians and pathologists, some favoring its inclusion among DLBCLs, others aiming to maintain it within the body of Burkitt's lymphoma. In the last version of the WHO Blue Book, it was felt relevant to include the term *Burkitt's-like lymphoma/atypical Burkitt's lymphoma*, in order to keep the process distinct from DLBCL due to the need for more aggressive therapy.[19] Like the *provisional entity* of the REAL Classification,[4] this represents a compromise, which may have an operational value, but neither solves the still open issue of the histogenesis of Burkitt's-like lymphoma nor provides definite criteria for its morphologic distinction from Burkitt's lymphoma on the one hand and DLBCL on the other.

At conventional light microscopy, neoplastic cells of Burkitt's-like lymphoma show significant variability in terms of shape and size, although the growth appears strongly basophilic with evident mitotic figures, apoptotic bodies and *starry-sky* pattern (Figure 5e). The diagnosis relays on rather subjective criteria, as also shown by a recent study carried out by the ILSG Members (*IX Meeting, Toulouse, October 11-13, 1999*). Immunohistochemistry reveals some phenotypic differences from Burkitt's lymphoma: in fact, staining for bcl-2 is positive, while strains for CD10 and bcl-6 provide negative results.[61] On the other hand, the determination of the Ki-67 proliferation-associated nuclear antigen shows that most if not all neoplastic cells are in the cell cycle, like those of Burkitt's lymphoma.[19] Cytogenetic studies reveal frequent occurrence of the same translocations as in Burkitt's lymphoma. This led someone to propose the detection of t(8;14), t(2;8) or t(8;22) as the key-marker for the recognition and assembly of this group of lymphoid tumors: recent studies, however, show

that these translocations can also occur in lymphomas which have completely different morphology, histogenesis and natural history.[55,57]

T/NK-cell lymphomas

Peripheral T/NK-cell lymphomas (PTCLs) represent about 10% of all lymphoid tumors in Western countries.[4,63-65] For practical purposes, they should be divided into anaplastic and non-anaplastic forms. The latter are rare clinicopathologic entities, which are characterized by marked cellular pleomorphism and occur at extranodal sites in at least 1/3 of cases (Figures 6a and 6b). Extranodal non-anaplastic PTCLs usually carry cytotoxic phenotype (TIA-1$^+$) with frequent co-expression of activation markers (granzyme-B$^+$, perforin$^+$) – a finding observed in only 20% of the nodal forms – and tend to develop in patients with an immunodeficient status, often following an organ transplantation.[65-78] Non-anaplastic PTCLs generally show defective T-cell antigen expression (CD2-CD8), mount α/β TCR (the γ/δ form being rarely observed and usually in hepatosplenic PTCL), display clonal rearrangements of the genes encoding for the T-cell receptor, and may be pathogenically related to a viral infection (such as HTLV1 for the adult T-cell lymphoma/leukemia occurring in Japan and EBV for the nasal T-cell lymphoma of Asian countries or AILD-type peripheral T-cell lymphoma) (Figure 6c).[4,64,65] On the whole, non-anaplastic PTCLs have a very poor clinical course in spite of the aggressive therapies employed (complete remission rate: 50%; overall survival at 5 years: 30-35%; disease-free survival at 5 years: about 20%).[63-65,79] These frustrating results might be partially due to the little attention paid to non-anaplastic PTCLs in Western countries, which has not favored the development of *ad hoc* therapies.

The anaplastic form of PTCL, usually termed *anaplastic large cell lymphoma* (ALCL), has been more extensively studied because of its relatively high frequency in Western countries and more favorable response to therapy (overall survival at 5 years: about 80%).[19,63,80-85]

ALCL was first described by Stein *et al*. in 1985 as a tumor characterized by large cells with a wide rim of cytoplasm (greyish-violet at Giemsa staining), variably shaped nuclei, prominent nucleoli, cohesive growth pattern, intrasinusoidal diffusion, and regular expression of the CD30 molecule (86) (Figures 7a and 7b). In 1988 it was included in the UKC among both B- and T-cell lymphomas.[2,3] Subsequent studies on larger series of cases led to the identification of clinico-pathologic variants: a) primary systemic (of the common, giant-cell rich, lympho-histiocytic, small cell, Hodgkin's-related/like, sarcomatoid, and signet ring cell types) (Figures 7c-j), b) primary cutaneous, and c) secondary.[85-94] In 1994, the REAL classification restricted the term *ALCL* to neoplasms with T- or null-phenotype and regarded the Hodgkin's-like (HL) variant as a provisional entity.[4,82,83,87] These concepts have been maintained in the recently developed WHO scheme[19] with two major differences: a) cutaneous ALCLs have been included among primary CD30$^+$ lymphoproliferative disorders of the skin,[68] and b) ALCL-HL has not been autonomously quoted because of the need for further studies. At the state of the art, primary nodal ALCL is regarded as a tumor with a very aggressive presentation, but also one which is very sensitive to chemotherapy.[13,63,80-85]

Over the last two years, the definition of ALCL has been further refined as the result of new findings, such as the expression of cytotoxic molecules and ALK protein.

About 80% of ALCLs display an activated cytotoxic phenotype (TIA-1$^+$, granzyme B$^+$, perforin$^+$), irrespectively of clinical and histologic features.[95-97] This feature represents an exception among nodal PTCLs, since the non-anaplastic forms reveal such a phenotype in no more than 20% of cases.[69] For the time being, the expression of cytotoxic markers has mere histogenetic relevance, and does not seem to affect the clinical course of the disease.

The expression of ALK protein is more often due to the occurrence of the (2;5)(p23;q35) translocation, which was described about 10 years ago as being characteristically associated with ALCL.[98] Subsequent molecular studies have shown that t(2;5) produces the formation of a hybrid gene, termed NPM/ALK, which encodes for a chimeric protein formed by the N-terminal region of nucleophosmin (numatrin/B23) and the entire cytoplasmic domain of the tyrosine kinase receptor ALK.[99-105] The development of specific antibodies against intracytoplasmic ALK domain and the N- and C-terminal regions of NPM, as well as their systemic application to large series of cases,[106-111] has revealed that:

1. about 60% of ALCLs do express the ALK protein;[85,106,108,109,111]
2. these cases correspond strictly to systemic forms, since cutaneous ALCLs are regularly negative:[85,112] in particular, ALK-positivity is observed in the vast majority of the tumors with common morphology and in most if not all lympho-histiocytic and small cell variants, while it is much rarer in the Hodgkin's-like and giant cell-rich forms (15-30%)[85,109-111] (Figures 7h, i and k);
3. the ALK staining usually occurs both in the nucleus and cytoplasm of the neoplastic ele-

ments, since the hybrid NPM/ALK protein forms a heterodimer with normal NPM, which is a *shuttle-protein* involved in the transcription mechanisms;[85,103,109,111]
4. the same nuclear and cytoplasmic staining pattern is also observed with the antibodies raised against the N-terminal region of NPM, but not with the ones specific for its C-terminal portion, which produce a nuclear positivity, since they react with normal NPM that is physiologically harvested within the nucleus;[85]
5. all ALK+ ALCLs carry t(2;5)(p23;q35);[85]
6. in a small number of ALCLs, ALK-positivity is limited to the cytoplasm (Figure 7l): this finding corresponds to the occurrence of chromosomal aberrations other than t(2;5), involving chromosome 2 at p23, such as inv(2)(p23;q35), t(1;2)(q21-25;p23), t(2;2)(p23;q23), or t(2;3) (p23;q21);[113-118]
7. ALK+ ALCLs invariably carry a T- or null-phenotype and result EMA$^+$ and CD15$^-$;[4,19,85,109,111]
8. besides the classic large cells, recently retermed *hallmark cells*, these tumors show a small-cell component, which is particularly evident in the lympho-histiocytic and small-cell variants and can vary during the course of the disease (for instance, at the time of presentation and relapse);[85,109,111]
9. ALK$^+$ ALCL is characterized by a spectrum of morphologic features which can reflect the ratio between large and small elements, the occurrence of fibrosis (which can be responsible for the HL appearance), and the presence of histiocytes or other reactive components, likely attracted by cytokines released by neoplastic cells;[85,109,111]
10. ALK positivity is never observed in B- and T-cell tumors other than ALCL, with the exception of a very rare plasmablastic tumor which shows cytoplasmic staining, but does not carry t(2;5) or variants;[119]
11. a search for the ALK protein in Hodgkin's lymphoma is invariably negative;[85,109,111]
12. ALK protein is not detected in normal lymphocytes, thus representing an important indicator both for diagnosis and detection of minimal residual disease;[85,107]
13. ALK$^+$ ALCLs more often affect patients under 30 years old and show a better response to therapy and more favorable clinical course than the ALK- ones.[84,110]

On the whole, the above mentioned findings strengthen the appropriateness of: a) restricting the term ALCL to tumors with T- or null-phenotype, b) keeping the cutaneous forms separate, and c) excluding any relationship between ALCL and Hodgkin's disease. Furthermore, they provide more precise and practical criteria to be applied to the so-called ALCL-HL, as stated in the last version of the WHO Blue Book:[19]

a) ALK$^+$ ALCL can occasionally show nodular aggregation and fibrosis, as seen in nodular sclerosing (NS)-HD;
b) NS-HD may be rich enough in neoplastic cells to be confused with ALCL;
c) in problematic cases, the expression of CD15, possibly in conjunction with positivity for B-cell markers, and the lack of TCR gene rearrangements and ALK protein favor the diagnosis of HD, while the negativity for CD15, the expression of T-cell markers and/or ALK protein, and the presence of TCR gene clonal rearrangements or NPM/ALK hybrid gene support the diagnosis of ALCL; cases which cannot be resolved by the combination of cell morphology, phenotype, and molecular data should be regarded as *unclassifiable* and submitted to a second biopsy or a treatment equally effective for ALCL and HD.[120]

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SAP was responsible for the conception and design of this review. SA and ES were responsible for drafting the article. GFO was responsible for the analysis and interpretation of morphologic data. SP and MP were responsible for analysis and interpretation of phenotypic data. PPP was responsible for analysis and interpretation of clinical data. BG was responsible for analysis and interpretation of molecular data. PLZ and LL were responsible for revising the article critically. BF approved the final version of the paper.

The criteria for the order of names were involvement in design and organization of the paper, laboratory research, analysis of clinical data and reviewing the paper. The order of the names was decided on the basis of each individual contribution to the above criteria.

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