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Malignant Lymphomas

The pathologist's view point. Part I – indolent 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 B-cell lymphomas with an indolent behavior, 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.**

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

Key words: indolent lymphoma, morphology, phenotype, genotype, behavior.

Since the 1970s, several very different classifications of malignant lymphomas have been used around the world.[1-10] The resulting lack of uniform diagnostic criteria for lymphoid tumors has given rise to considerable problems both for pathologists and clinicians, and has seriously hampered comparison of the studies reported in the literature.[11-17] In theory, as with all other tumor types, lymphomas should be classified on the basis of their supposed histogenesis so as to provide maximum information on their biology, natural history and response to therapy. In practice, however, since our knowledge of the immune system is still insufficient for this approach to be applied in all cases, a *biologically correct* lymphoma classification is not currently feasible. Despite this, many hematopathologists agree that by pooling morphologic, immunophenotypic, molecular, and clinical findings it is possible to enumerate a large series of distinct entities that can be recognized and diagnosed in routine practice. In 1994, on the basis of the work of the *International Lymphoma Study Group* (ILSG), a list of "real", clearly characterized anatomo-clinical entities that can be readily recognized with currently available techniques was published as a proposal for an up-to-date practical classification of malignant lymphomas: this list was termed the *Revised European American Lymphoma* (REAL) Classification (Table 1).[18] This classification has recently been adopted by the *World Health Organization* (WHO) as an operative guideline for studying and diagnosing malignant lymphomas; furthermore, its methodology has been extended to the categorization of hematopoietic non-lymphoid tumors.[19]

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Histologic grade and clinical aggressiveness

Before the advent of immunophenotyping and molecular biology techniques that have allowed the identification of many lymphoid neoplasms as separate entities, it was thought that non-Hodgkin's lymphoma constituted a single generic disease with various degrees of aggressiveness that could be revealed on the basis of morphology and clinical findings. This concept encouraged the convinction that it should be possible to devise a single grading system capable of predicting the clinical course of the disease. This was the principle behind the Working Formulation,[8] in which lymphoid tumors were divided into three prognostic groups (indicated by grades of clinical malignancy) on the basis of the survival of the patients recruited in the original study. Nevertheless, each of these wide-ranging categories is actually known to contain a large number of conditions that differ greatly as regards their eti-

Table 1. The REAL classification in the form adopted by the WHO.

B-cell neoplasms

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

Mature (peripheral) B-cell neoplasms
- B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma
- B-cell prolymphocytic leukemia
- Lymphoplasmacytic lymphoma
- Splenic marginal zone B-cell lymphoma (± villous lymphocytes)
- Hairy cell leukemia
- Plasma cell myeloma/plasmacytoma
- Extranodal marginal zone B-cell lymphoma of MALT type
- Mantle cell lymphoma
- Follicular lymphoma
- Nodal marginal zone B-cell lymphoma (± monocytoid B cells)
- Diffuse large B-cell lymphoma
- Burkitt's lymphoma/Burkitt cell leukemia

T- and NK-cell neoplasms

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

Mature (peripheral) T-cell neoplasms
- T-cell prolymphocytic leukemia
- T-cell granular lymphocyte leukemia
- Aggressive NK-cell leukemia
- Adult T-cell lymphoma/leukemia (HTLV1+)
- Extranodal NK/T-cell lymphoma, nasal type
- Enteropathy-type T-cell lymphoma
- Hepatosplenic γδ T-cell lymphoma
- Subcutaneous panniculitis-like T-cell lymphoma
- Mycosis fungoides/Sézary syndrome
- Anaplastic large cell lymphoma T/Null cell, primary cutaneous type
- Peripheral T-cell lymphomas, not otherwise specified (NOS)
- Angioimmunoblastic T-cell lymphoma
- Anaplastic large cell lymphoma T/Null cell, primary systemic type

Hodgkin's disease (Hodgkin's lymphoma)

- Nodular lymphocyte predominance Hodgkin's disease
- Classical Hodgkin's disease
  - Nodular sclerosis Hodgkin's disease
  - Lymphocyte-rich classical Hodgkin's disease
  - Mixed cellularity Hodgkin's disease
  - Lymphocyte depletion Hodgkin's disease

Only major categories are included.

ology, presentation, natural history, epidemiology and response to treatment. Moreover, each single variety of lymphoma displays its own spectrum of degrees of morphologic and clinical aggressiveness. As a result, it no longer appears possible to categorize lymphoid tumors on the basis of a generic grading system that would be tantamount to considering as a single entity different types of lung cancer, such as the carcinoid, squamous cell carcinoma, adenocarcinoma and small cell carcinoma. Nor can the degree of malignancy of a lymphoma be realistically determined on the basis of cell size, as was envisaged by the *Updated Kiel Classification* (UKC).[9,10] Indeed, this principle would lead to mantle cell lymphomas and anaplastic large cell lymphomas (ALCL) being interpreted, respectively, as low and high grade forms, in exact contrast to the findings of a validation study on the REAL Classification promoted by the *U.S. National Cancer Institute* and *S. Salvatore Foundation* in March 1994, which demonstrated that mantle cell lymphomas are associated with a 5-year survival rate of less than 30%, while that of ALCL is about 80%.[20]

Clinical categorization of non-Hodgkin's lymphomas

The large series of different entities that can be distinguished on morphologic, immunophenotypic and biological grounds and that are generally included under the umbrella term non-Hodgkin's lymphoma can be ordered on the basis of various principles, including their supposed normal counterpart within the immune system, their morphologic appearance and their clinical characteristics. For the practical oncologist, the most rational criterion is their predictable behavior. Thus, patients with lymphoid tumors can be divided into two different main groups on the basis of the characteristics of the process at the time of presentation and the patients' life expectancy, as proposed by Longo *et al.*: indolent and aggressive lymphomas.[21,22] At times, a further distinction between aggressive and very aggressive lymphomas is made, depending on the expected survival - weeks or months - in untreated cases. This clinical grouping is not mentioned in the REAL Classification. In fact, on the basis of what has been reported in the literature, the ILSG members agreed that the aggressiveness of malignant lymphomas varies significantly among different histologic categories and within each category among different patients. Such a variability is due to the influence exerted - singly or in combination - by biological factors, such as cytokinesis (i.e. cell proliferation and loss), oncogene activation, presence of hybrid fusion genes, development of multidrug resistance, microambient, or correlation with micro-organisms (e.g. *Helicobacter pylori* and hepatitis C virus).[23-34] For all these reasons, the ILSG members preferred to avoid the attribution of grades of malignancy, thinking that they cannot be defined by pure morphology or statistical analysis: the latter only informs on the natural history of the disease or on its median response to treatment and thus is neither predictive of the single patient's outcome nor supports *ad hoc* therapeutic decisions (so-called tailored therapy).[35,36] These concepts found substantial confirmation at the *VII International Conference on Malignant Lymphomas* (Lugano, 1-5 June 1999).[37,38] In particular, 1,093 cases were gathered into indolent, aggressive and very aggressive lymphomas according to the above mentioned

clinical criteria.[38] When the outcome within each group was evaluated on the basis of individual histologic subtypes, it emerged that clinical grouping is a rather rough tool: for instance, among aggressive lymphomas, the 5-year survival rates ranged from 78% for anaplastic large cell lymphomas to 14% for mantle cell lymphomas, with intermediate rates of 38% for diffuse large B-cell lymphomas and 68% for follicular lymphomas grade 3.[38]

Indolent lymphomas

Bearing in mind the above mentioned limitations on the absolute value of the terms *indolent* and *aggressive*, in the clinical setting indolent lymphomas are considered to be those associated with a survival measurable in years, independently of whether or not any therapy is applied. These lymphoproliferative disorders have very variable clinical presentations. Some are constantly systemic diseases, often with leukemic manifestations. Others have an extranodal primary presentation and can remain localized for long periods, even in the absence of any therapy. Yet others correspond to tumors with nodal presentation, which can have widespread immune system involvement at the time of diagnosis. This leads to the basic distinction of three fundamental subtypes of indolent lymphoma: disseminated leukemias/lymphomas, extranodal forms and nodal ones. On pathologic grounds, indolent lymphomas display *low grade* histologic characteristics with a strong prevalence of small cells and a blastic ratio of less than 25% of the total population. Furthermore, the number of mitotic figures is low, according to the criteria of the UKC,[9,10] and constantly less than 5 mitoses per high-power field. A common feature of many different histologic types of indolent lymphoma is the tendency to undergo histologic transformation into a high-grade form, with a corresponding acceleration of the clinical course. In the following the morphologic characteristics of B-cell indolent lymphomas – excluding plasmacytoma/plasma cell myeloma – will be described; the subclassification is reported in Table 2.

Table 2. List of indolent B-cell lymphomas.

Disseminated lymphomas/leukemias
- B-cell chronic lymphocytic leukemia
- Lymphoplasmacytic lymphoma
- Hairy cell leukemia
- Splenic marginal zone B-cell lymphoma (± villous lymphocytes)
- Plasma cell myeloma/plasmacytoma

Extranodal lymphomas
- Extranodal marginal zone B-cell lymphoma of MALT type

Nodal lymphomas
- Small lymphocytic lymphoma
- Follicular lymphoma
- Nodal marginal zone B-cell lymphoma (± monocytoid B cells)

B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma

B-cell chronic lymphocytic leukemia (B-CLL) is a well-known process, whose incidence is often underestimated by pathologists, since the diagnosis is more often based on the examination of peripheral-blood and/or bone marrow smears.[7,10,18] However, histopathology largely concurs to tumor staging and aggressiveness definition by showing the exact amount of bone marrow infiltrate, cellular composition and growth pattern.[7,10,18] B-cell small lymphocytic lymphoma (B-SLL) is the solid equivalent of B-CLL:[18,39] it usually presents in the lymph node, but can infiltrate the bone marrow, also in the absence of an overt peripheral blood spread. Morphologically,[7,10,18,40,41] B-CLL/SLL is mainly constituted by small lymphoid elements, with the contemporary presence of variable amounts of prolymphocytes and paraimmunoblasts (Figure 1a), that represent the proliferating compartment of the process and which in some areas can be numerous enough to give rise to pseudofollicles (Figure 1b). These latter – detectable only in tissue sections – seem to represent a worse prognostic indicator. In addition, prolymphocytes and paraimmunoblasts are extremely useful for the correct interpretation of inadequately fixed samples, when small lymphocytes assume a cleaved nuclear profile, which can lead to the wrong diagnosis of mantle-cell lymphoma. In the REAL Classification,[18] the B-CLL/SLL category includes forms with features of plasmacytoid differentiation (Figure 1c) and listed in the UKC as *lymphoplasmacytoid immunocytomas*. These forms, in fact, do not display significant clinical, prognostic, morphologic or phenotypic differences that can justify a clear-cut distinction from typical B-CLL.[42] B-CLL also includes the rarer forms of B-prolymphocytic leukemia (B-PLL): this decision is based on the observation that over time B-CLL tends to enrich itself in prolymphocytic forms (the so-called prolymphocytoid crisis) (Figure 1d), in such a way that – even when FAB criteria[40] are applied – the distinction between the two leukemic forms can become quite arbitrary.[39,43] B-CLL and B-PLL probably represent the two extremes of a single disease, provided with different degrees of aggressiveness. In the WHO scheme,[19] however, B-CLL and B-PLL are listed separately. Finally, a diffuse large B-cell lymphoma (DLBCL) can occasionally develop within the context of B-CLL/SLL. Such an event is

Table 3. Phenotypic profile of 90 indolent B-cell lymphomas.

	CD3	*CD5*	*CD23*	*CD10*	*CD20*	*CD79a*	*Bcl-2*	*Bcl-6*	*Bcl-1*	*IRF4*	*BSAP*
B-CLL	– (20/20)	+ (20/20)	+ (20/20)	– (20/20)	+ (20/20@)	+ (20/20)	+ (20/20)	– (20/20)	– (20/20*)	+ (19/19@)	+ (15/15)
LPL	– (15/15)	– (15/15)	+ (3/15)	– (15/15)	+ (15/15)	+ (15/15)	+ (15/15)	– (15/15)	– (15/15)	+ (15/15)	+ (15/15)
MCL	– (10/10)	+ (10/10)	– (10/10)	– (10/10)	+ (10/10)	+ (10/10)	+ (10/10)	– (10/10)	+ (10/10)	– (8/10§)	+ (9/9)
FCL	– (20/20)	– (20/20)	+ (4/20)	+ (20/20)	+ (20/20)	+ (20/20)	+ (20/20)	+ (19/19)	– (20/20)	– (6/20#)	+ (15/16)
MZL/E	– (10/10)	– (10/10)	– (10/10)	– (10/10)	+ (10/10)	+ (10/10)	+ (8/8)	– (10/10)	– (10/10)	–/+w (7/7)	–/+w (7/7)
MZL/N	– (4/4)	– (4/4)	– (4/4)	– (4/4)	+ (4/4)	+ (4/4)	+ (4/4)	– (4/4)	– (4/4)	–/+w (3/3)	–/+w (4/4)
MZL/S	– (6/6)	– (6/6)	– (6/6)	– (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	– (6/6)	– (4/4)	– (4/4)	+ (6/6)
HCL	– (5/5)	– (5/5)	– (5/5)	– (5/5)	+ (5/5)	+ (5/5)	+ (5/5)	– (5/5)	– (5/5)	– (5/5)	+ (3/5)

Abbreviations: B–CLL: B–cell chronic lymphocytic leukemia. LPL: Lymphoplasmacytic lymphoma. FCL: Follicle center cell lymphoma. MZL/E: Marginal zone lymphoma, extranodal. MZL/N: Marginal zone lymphoma, nodal. MZL/S: Marginal zone lymphoma, splenic. HCL: Hairy cell leukemia. +More than 75% of neoplastic cells positive. +/–: 50–75% of neoplastic cells positive. –/+: 25–50% of neoplastic cells positive. —Neoplastic cells virtually negative. @Small lymphoid elements are weakly stained, if stained; prolymphocytes and paraimmunoblasts of pseudofollicular structures are strongly positive. §Scattered positive cells. #Positive only in cases with plasmacellular differentiation. W: weak staining.

commonly termed Richter's syndrome and its mechanisms are still controversial: in fact, in some cases the supervening large B-cell tumor represents the blastic transformation of the same B-CLL clone, while in others it corresponds to a *de novo* neoplasm, whose occurrence might be facilitated by the defective immune response of B-CLL patients.[18] On phenotypic grounds (Table 3), B-CLL is characterized by the expression of B-cell markers, such as CD19 (often weak), CD22, and CD79a.[18] Conversely to that which is observed in other B-cell tumors, the small cell component is very weakly stained for CD20 in tissue sections, prolymphocytes and paraimmunoblasts representing the only consistently positive components (Figure 1e).[44] This finding seems clinically relevant in the light of the increasing therapeutic usage of anti-CD20 antibodies.[45] The profile of the tumor is further characterized by the presence of CD5 and CD23 at the cell membrane level (Figures 1f and 1g). As these molecules may be expressed at different densities and are rather sensitive to fixation, their detection is greatly facilitated by the application of effective antigen retrieval techniques.[46] In particular, CD5 and CD23 along with other markers allow the distinction of B-CLL/SLL from other lymphoid tumors with different origin and behavior (Table 1), such as mantle-cell lymphoma (MCL) ($CD5^+$, $CD10^-$, $CD23^-$, $DBA.44^-$, $CD68^-$), immunocytoma (IC) ($CD5^-$, $CD10^-$, $CD23^{+/-}$, $DBA.44^-$, $CD68^-$), marginal-zone lymphoma (MZL) ($CD5^-$, $CD10^-$, $CD23^{-/+}$, $DBA.44^{-/+}$, $CD68^{+/-}$), hairy cell leukemia (HCL) ($CD5^-$, $CD10^-$, $CD23^{-/+}$, $DBA.44^+$, $CD68^{+/-}$), and follicle center lymphoma (FCL) ($CD5^-$, $CD10^+$, $CD23^-$, $DBA.44^-$, $CD68^-$). As far as concerns B-CLL, CD23 expression is indeed much stronger in prolymphocytes and paraimmunoblasts than in the small cell component (Figure 1g):[47,48] this finding seems to have prognostic relevance, the cases with a high content of $CD23^+$ elements running a more aggressive clinical course. Immunoglobulin (Ig) expression is exceedingly weak, thus preventing its detection on tissue sections in most instances. However, cases with plasmacytoid differentiation are characterized by a more abundant Ig production, with detectable amounts at the intracytoplasmic level. More recently, some additional markers have been proposed and found useful for the diagnosis of B-CLL/SLL, such as cyclin D1, bcl-2 protein, bcl-6 product, multiple myeloma oncogene 1/interferon regulatory factor-4 (MUM1/IRF4), and PAX-5 gene product/B cell-specific activator protein (PAX5/BSAP).[18,49-51] The search for cyclin D1 is often employed in the differential diagnosis between B-CLL/SLL and MCL, since the latter shows regular overexpression of the molecule due to the occurrence of t(11;14) or bcl-1 gene rearrangement.[18,52] On rare occasions, however, cyclin D1 positivity can also occur in B-CLL, a fact that strengthens the relevance of CD23 detection.[53] The bcl-2 product is always strongly expressed by B-CLL/SLL: this finding does not correspond to the presence of t(14;18), but indicates a certain protection of neoplastic cells from apoptosis.[18] The latter finding along with the low proliferative activity (as shown by the Ki-67 marking) is responsible for the typical slow progression of the tumor. Bcl-6 is never expressed by lymphomatous cells:[49] its presence or absence is very useful for distinguishing between neoplastic pseudofollicles ($bcl\text{-}6^-$, $CD10^-$, $bcl\text{-}2^+$, $CD5^+$, $CD23^+$) and residual germinal centers ($bcl\text{-}6^+$, $CD10^+$, $bcl\text{-}2^-$, $CD5^-$, $CD23^-$). The application of the newly developed antibodies raised against the transcription factors IRF4 and BSAP produce opposite patterns.[50,51] Small lymphoid elements are $BSAP^+$

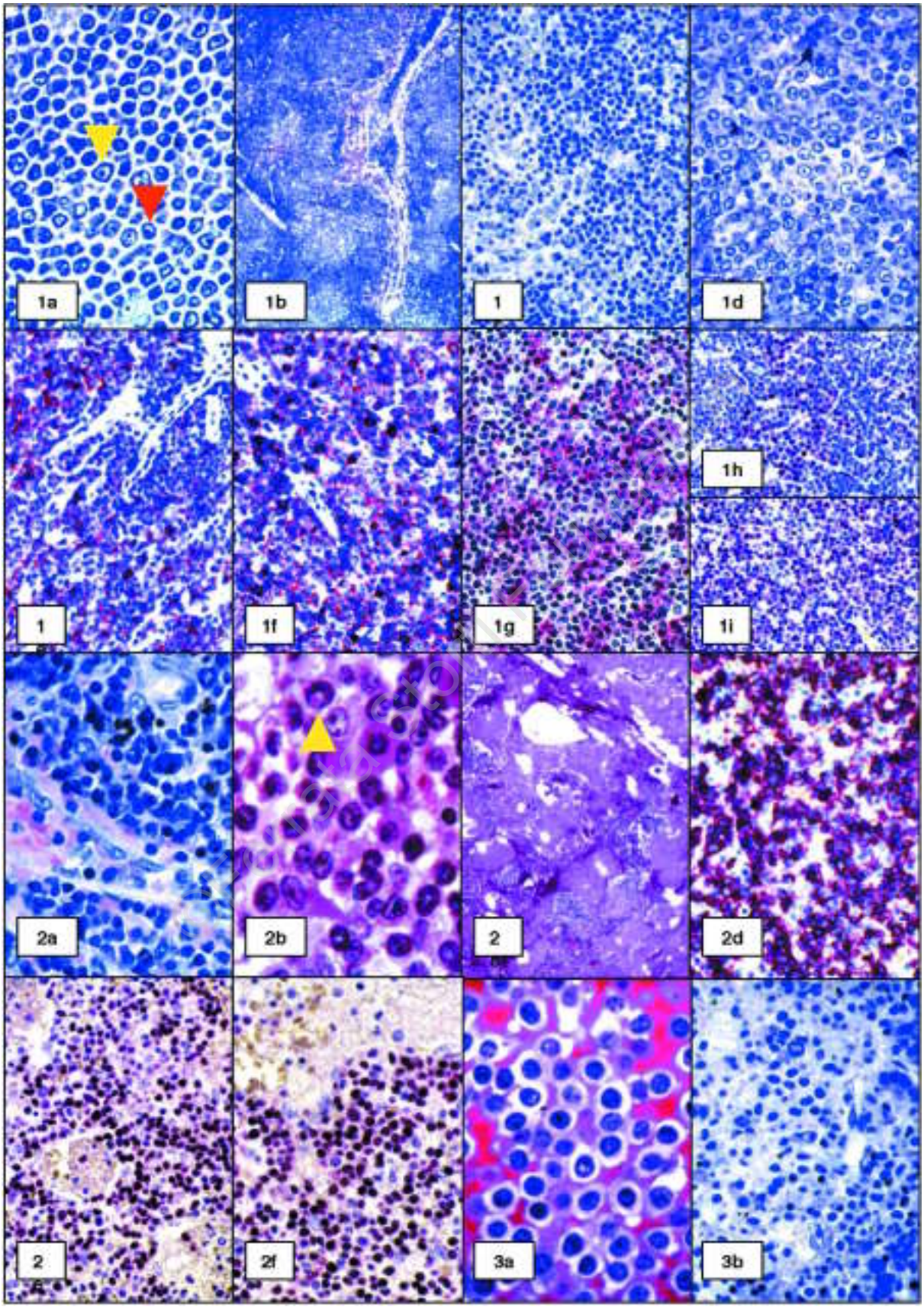
and IRF4$^-$, while prolymphocytes and paraimmunoblasts (as easily seen in pseudofollicles) appear BSAP$^{-/+}$ and IRF4$^+$ (Figures 1h and 1i). The latter finding – detected by our group in large series of cases – fits with the recent observation that more than 50% of B-CLL cases might be derived from memory B-cells as suggested by the occurrence of bcl-6 and IgV gene mutations.[54-57] In fact, IRF4 – that is the product of the MUM-1 gene – is physiologically expressed by B-lymphocytes following germinal center cell selection (i.e. by some centrocytes in the light zone of the germinal center, plasmacytoid elements and plasma cells). Cases without bcl-6 and IgV gene mutations might stem from naive B-cells, do express CD38, carry trisomy 12, occur more often in males and run a rather aggressive clinical course.[54-57] Cytogenetic aberrations other than trisomy 12 are encountered and consist in chromosome 17 abnormalities,[58] 14q$^+$, and a deletion in the 13q14 chromosomal region, involving a not yet identified tumor suppressor gene and occurring in approximately 60% of B-CLL cases.[59] At the molecular level, mutation or loss of heterozygosity of the p53 gene usually occurs in association with Richter's syndrome.[59] C-myc, bcl-1, and bcl-2 genes have not been found to be clearly associated with the disease, although t(11;14) has occasionally been observed in "atypical" B-CLL.[60,61]

Lymphoplasmacytic lymphoma

This has practically become a category by way of exclusion.[18] In fact, it comprises neoplasms that do not display characteristics that would allow their inclusion among B-CLL/SLL, MCL, MZL or FCL.[18] The immunocytoma of the REAL classification[18] – termed only lymphoplasmacytic lymphoma (LPL) in the WHO scheme[19] – actually corresponds to the *lymphoplasmacytic immunocytoma* of the UKC,[9,10] since it is made up of elements that range from small lymphocytes to mature plasma cells by way of lymphoplasmacytoid forms (Figure 2a). The production and accumulation of intracytoplasmic Ig leads to the formation of frequent hyaline inclusions in the form of Russell's and/or Dutcher's bodies (Figure 2b)[7,10,18] and, on occasions, to phenomena of crystallization and phagocytosis by reactive histiocytes.[62] On clinical grounds, the tumor is more aggressive than that of B-CLL/SLL,[63] may transform into a diffuse large B-cell lymphoma (immunoblastic)[64] and frequently shows features corresponding to the original description of Waldenström's disease,[65] such as diffuse bone marrow involvement and a monoclonal IgM/κ component in the serum. The latter may produce a hyperviscosity syndrome or autoimmune phenomena, more frequently in the form of hemolytic anemia. On occasions, LPL gives rise to amyloid deposits in several organs and apparatuses (Figure 2c):[66-68] in the heart, these can cause severe arrhythmia with possible sudden death.[69] At immunohistochemistry (Table 3), neoplastic cells strongly express B-cell markers, including CD20 (Figure 2d), while they regularly lack CD5, CD10 and CD68.[18] CD23 is found in a proportion of cases.[18] Monotypic immunoglobulins are present at high density both at the surface and intracytoplasmic level.[18] The bcl-2 gene product is expressed independently of the occurrence of t(14;18) causing protection against apoptosis. The Ki-67 marking is usually low, with the exception of the cases that undergo blastic transformation. The search for the bcl-6 protein gives negative results in the neoplastic component, while it allows easy detection of residual germinal centers, which in turn are bcl-2$^-$.[49,70] Cyclin D1 is never overexpressed.[52] Neoplastic cells carry BSAP and IRF4, the latter matching with plasmacytoid/plasmacellular differentiation (Figures 2e and 2f).[50,51] The above mentioned phenotypic profile differs significantly from that of other small B-cell lymphoid tumors, thus representing a basic tool for LPL recognition. Cytogenetic and molecular biology studies have shown the occurrence of t(9;14)(p13;q32) in about 50% of cases.[71] The chromosomal breakpoints of the translocation involve the IgH locus on chromosome 14q32 and – on chromosome 9p13 – the genomic region containing the PAX-5 (paired homeobox-5) gene. Since this gene encodes BSAP, it is not surprising to find expression of this molecule in LPL, which might contribute to tumor development.[51] Recently, a 7q deletion has been described in small cell lymphomas with immunocytic morphology.[72]

Hairy cell leukemia

Hairy cell leukemia (HCL) consists morphologically of small-medium sized B-cells, with variably shaped, round, oval or cleaved nuclei with a fairly wide cytoplasmic rim provided with the characteristic villous projections that give the form its name[7,10,18] (Figure 3a). Occasionally, neoplastic cells are a bit larger and show a small, but distinct central nucleolus: this condition is commonly called HCL variant and seems to have a more aggressive clinical course.[73,74] The neoplastic population is generally confined to the peripheral blood, bone marrow and the red pulp of the spleen, while the lymph nodes are only very rarely involved.[7,10,18] In the bone marrow, hairy cells produce interstitial infiltration with progressive replacement of normal hematopoietic series and low cellular density, due to the wideness of their cytoplasm (Figure 3b). Phenomena of edema and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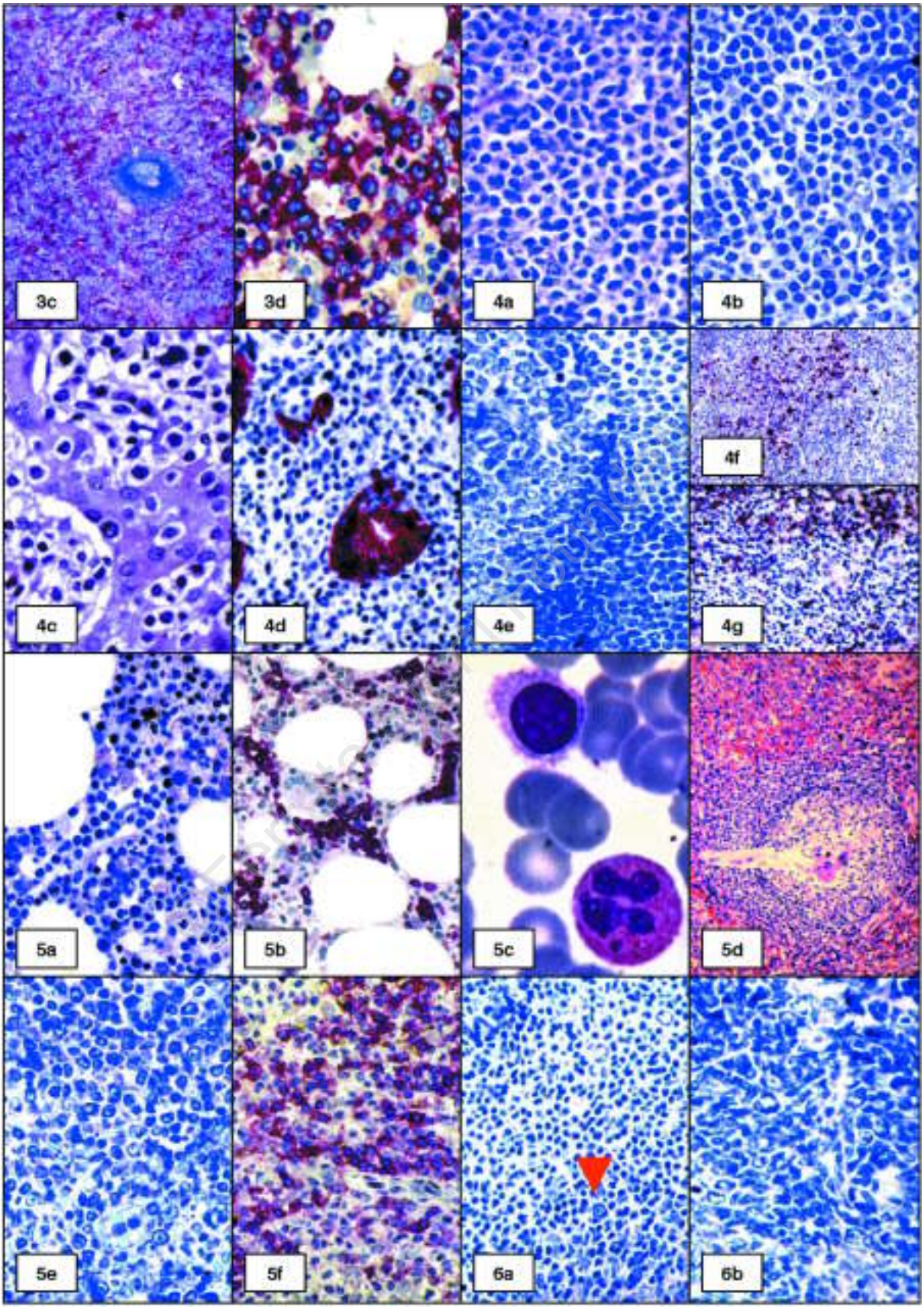
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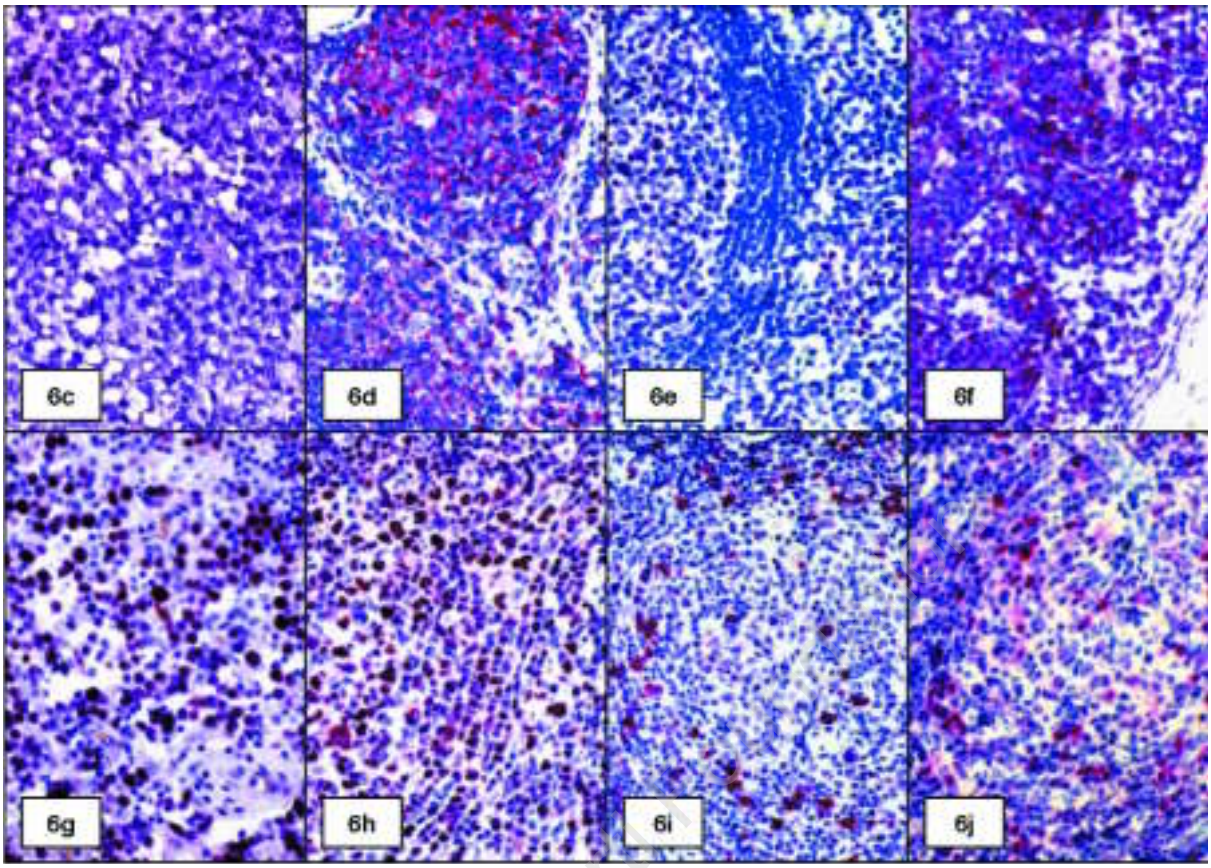

Figure 1 (page 1296). B-cell chronic lymphocytic leukemia / small lymphocytic lymphoma: a) cellular composition; the red and yellow arrows indicate a prolymphocyte and a paraimmunoblast, respectively (Giemsa; ×600); b) pseudofollicular growth pattern (Giemsa; ×50); c) features of lymphoplasmacytoid differentiation (Giemsa; ×300); d) prolymphocytoid crisis (Giemsa; ×600); e) the expression of CD20 is dim in small lymphocytes and strong in prolymphocytes and paraimmunoblasts (immunoalkaline phosphatase technique in paraffin sections; ×250); f) neoplastic cells are positive at the determination of CD5 (immunoalkaline phosphatase technique in paraffin sections; ×250); g) CD23 staining is much stronger in prolymphocytes and paraimmunoblasts (immunoalkaline phosphatase technique in paraffin sections; ×300); h) BSAP expression is limited to pseudofollicles (immunoalkaline phosphatase technique in paraffin sections; ×150); i) IRF4 positivity is mostly expressed by small lymphocytes (immunoalkaline phosphatase technique in paraffin sections; ×150).

Figure 2 (page 1296). Lymphoplasmacytic lymphoma: a) the tumor consists of small lymphocytes, plasmacytoid elements and plasma cells (Giemsa; ×600); b) a Dutcher's body (arrowed) appears in the form of an "intranuclear inclusion" (P.A.S.; ×800); c) amyloid deposits in a lymph node (hematoxylin and eosin; ×50); d) CD20 is strongly expressed (immunoalkaline phosphatase technique in paraffin sections; ×200); e) all neoplastic cells display strong positivity at BSAP determination (immunoalkaline phosphatase technique in paraffin sections from a bone marrow biopsy; ×300); f) the search for IRF4 produces a similar staining pattern (immunoalkaline phosphatase technique in paraffin sections from a bone marrow biopsy; ×400).

Figure 3. (pages 1296 and 1297). Hairy cell leukemia: a) neoplastic cells show variability of the nuclear profile and a rather wide rim of clear cytoplasm (hematoxylin and eosin; ×800); b) bone-marrow interstitial infiltration by hairy cells; note the low cellular density (Giemsa; ×300); c) neoplastic elements infiltrate the red pulp, sparing Malpighian corpuscles (Giemsa; ×50); d) DBA.44 staining highlights the typical "hairy" cytoplasmic profile, thus allowing the easy identification and counting of neoplastic cells (immunoalkaline phosphatase technique in paraffin sections; ×600).

Figure 4. (page 1297). Extranodal marginal zone B-cell lymphoma: a) cytological details: centrocyte-like variant (Giemsa; ×400); b) cytological details: monocytoid-like variant (Giemsa; ×400); c) lympho-epithelial lesions (hematoxylin and eosin; ×600); d) the same as negatively shown by the application of the anti-cytokeratin antibody MNF.116 (immunoalkaline phosphatase technique in paraffin sections; ×300); e) features of follicular colonization (Giemsa; ×400); f) the staining for bcl-6 reveals residual follicle center cells (immunoalkaline phosphatase technique in paraffin sections; ×100); g) content of $CD4^+$ cells in a gastric marginal zone lymphoma of the small cell type (immunoalkaline phosphatase technique in paraffin sections; ×100).

Figure 5. (page 1297). Splenic marginal zone B-cell lymphoma: a) the exact amount of neoplastic cells and their intrasinusoidal distribution are not easily assessed in conventionally stained preparations (Giemsa; ×500). b) CD20 immunostaining allows the clear-cut assessment of the tumor burden and distribution (immunoalkaline phosphatase technique in paraffin sections; ×300); c) circulating neoplastic cells show the villous profile (May-Grünwald-Giemsa; ×800); d) neoplastic cells grow around residual Malpighian corpuscles and infiltrate the red pulp (Giemsa; ×100); e) the tumors consists of small elements with monocytoid appearance, intermingled with some plasma cells and blasts (Giemsa; ×400); f) IgD staining of a bone marrow biopsy (immunoalkaline phosphatase technique in paraffin sections; ×400).

Figure 6. (page 1297 and this one). Follicle center lymphoma: a) grade I form: note the follicular aggregation and very low content of centroblasts (one of which is arrowed) (Giemsa, ×300); b) grade II form: the content of centroblasts exceeds the value of 6 / HPF (Giemsa, ×400); (page 1297). c) follicle center lymphoma with a high content of signet-ring-like elements (hematoxylin and eosin, ×400); d) CD10 expression by neoplastic cells; note the presence of stained elements in an interfollicular position (immunoalkaline phosphatase technique in paraffin sections; ×250); e) the same with the anti-bcl-6 PG-B6 monoclonal antibody (immunoalkaline phosphatase technique in paraffin sections; ×250); f) overexpression of the bcl-2 product (immunoalkaline phosphatase technique in paraffin sections; ×250); g) high Ki-67 marking in a diffuse form (immunoalkaline phosphatase technique in paraffin sections; ×250); h) IRF4 antigen expression by neoplastic cells; note the presence of stained elements in an interfollicular position (immunoalkaline phosphatase technique in paraffin sections; ×250); i) follicle center lymphoma with features of plasma cell differentiation, as shown by the determination of kappa Ig light chain (immunoalkaline phosphatase technique in paraffin sections; ×250); j) the same field at the search for BSAP (immunoalkaline phosphatase technique in paraffin sections; ×250).

hemorrhage are frequently seen. In the spleen, the hairy cells substitute the red pulp with pseudosinus formation (Figure 3c) and spare Malpighi's corpuscles for a long time. In addition to typical B-cell antigens, hairy cells express the receptor for interleukin 2 (CD25) and the CD103 integrin (a cell-adhesion molecule).[18,75] Unfortunately, these antigens are negatively affected by routine histopathology technical procedures. In paraffin sections, as well as being positive for pan-B markers such as CD20 and CD79a, the neoplastic cells often express the CD68 molecule and are labeled by antibody DBA.44 (Figure 3d).[76-78] The latter highlights the typical hairy profile of neoplastic cells, which otherwise is hardly visible. DBA.44, however, does not react or only minimally reacts with the rare cases of HCL variant. Immunoglobulins are rarely detected in routine samples and, when detected, do not contain δ-heavy chain. The bcl-2 product is regularly expressed, BSAP is found in the majority of cases, while the search for bcl-1, bcl-6 and IRF4 always gives negative results (Table 3).[49-53] The Ki-67 marking is extremely low, a finding in keeping with the very indolent behavior of the process. More recently, monoclonal antibodies specific to human tartrate-resistant acid phosphatase have been produced and proposed as specific HCL markers.[79] HCL represents one of the tumors for which it is of fundamental importance to monitor minimal residual disease (MRD) following therapy: this goal can be easily achieved by the cheap immunohistochemical assay in paraffin sections. Indeed, patients treated with the more recent approaches such as interferons, 2-chlorodeoxyadenosine or deoxycoformycin have been shown to retain isolated residual hairy cells trapped within hyperplastic or fibrotic marrow, the recognition of which is difficult or even impossible at pure morphologic evaluation.[76-78,80,81] Molecular analysis shows a high rate of somatic mutations.[82] This finding does not completely clarify the exact position of HCL in B-cell development: it does, however, indicate that neoplastic cells originate from elements which have been exposed to the hypermutation mechanism and have thus passed through the germinal center.[82] Occasionally, genomic alterations have been described in the form of t(2;8), p53 gene deletion and trisomy 12.[83,84]

Extranodal marginal zone B-cell lymphoma of MALT type

This is a new category, which has been introduced by the REAL Classification[19] and maintained in the WHO scheme.[19] Histogenetically, the tumor can be traced to elements of the marginal zone surrounding the mantles of normal follicles, which is scarcely perceptible in the lymph nodes and clearly evident in Malpighian bodies in the spleen. B-cell lymphomas of the marginal zone can be divided into three categories: extranodal, nodal and splenic. The extranodal forms correspond to the mucosa-associated lymphoid tissue (MALT) lymphomas described in the early eighties by Isaacson.[85,86] These are primary B-cell lymphomas most frequently found in the stomach, intestine, salivary gland, lung, respiratory airway, thyroid, ocular adnexa and skin.[87-90] It is interesting to note that, with the exception of the intestine where MALT is normally present as Peyer's patches,[91] in the other sites the lymphoid tissue appears in an acquired form following infective or more often autoimmune inflammatory processes.[87-90] Neoplastic elements are usually small, have abundant cytoplasm and a variable nuclear profile, at times resembling centrocytes (cc-like cells), at times immunocytes (Ic-like cells) or B-monocytoid elements (Figures 4a and 4b).[18,85-90] The tumor shows regular plasma cell differentiation, which at times is associated with amyloid deposit[92] or is so striking as to lead to a misdiagnosis of extramedullary plasmacytoma.[88] The number of mitotic figures is low. Lymphomatous cells usually attack the epithelial component – giving rise to lympho-epithelial lesions (LELs) – and surround and colonize pre-existing follicles (Figures 4c-4e).[87-90,93] These findings are of value in recognizing extranodal MZLs. In the gastro-intestinal (GI) tract, the diagnosis made on small biopsies performed during endoscopy does not provide definite hints on the degree of GI wall infiltration, which should be assessed by other means, such as echo-endoscopy. In the stomach, where the tumor develops within the context of *Helicobacter pylori*+ (HP) chronic gastritis,[94,95] Wotherspoon *et al.* proposed adopting a score system which highlights morphologically suspicious lesions (grades III and IV), which do not yet represent overt lymphomas, but require careful follow-up with frequent biopsies.[94] MZL most often has a multicenter distribution within the organ of origin,[96] a fact that should always be considered when taking therapeutic decisions (e.g. surgery vs. chemotherapy). In case of dissemination to local nodes[88-90] and spleen,[97] the tumor frequently involves the marginal zone, sometimes without total effacement of the normal structure.[90] More rarely it colonizes other MALT sites (usually many years following the original diagnosis) or the bone marrow (reported incidence in different series: 5-10% of cases).[33] Recently, the ILSG members stated that the term MZL should be restricted to neoplasms consisting almost exclusively of small cells.[19] However, at MALT sites large B-cell lymphomas with or without residual MZL do also

occur, as do MZLs with a certain number of blasts.[98] The former situation should be diagnosed as DLBCL (with residual MZL, if present), while the latter is still matter of debate. Recent data from the *International Extranodal Lymphoma Study Group* (IELSG) (Ascona, February 25-26, 2000) suggest that a blastic component not exceeding 10% of the examined population does not affect the course of the disease. Further studies are needed to assess whether the cases with a higher number of blasts or clusters consisting of 20 or more blasts do actually run more aggressively – as proposed by Isaacson *et al.* – or not. At phenotypic analysis (Table 3),[18,33,88-90] neoplastic cells are $CD19^{-}CD22^{+}$, $CD79a^{+}$, $CD35^{+}$, $CD11c^{+}$, $CD68^{+/-}$, and $DBA.44^{-/+}$. They express monotypic Ig at the intracytoplasmic (perinuclear) level, bearing μ or – more rarely – α heavy chain, but not δ chain. There is weak-moderate positivity at the determination of the bcl-2 gene product. The search for CD5, CD10, CD23, bcl-1, and bcl-6 turns out to be negative (Figure 4f). The content of Ki-67^{+} cells is low. IRF4 and BSAP are detected in a proportion of the neoplastic cells.[50,51] T-cell markers reveal the presence of a high content of reactive T-lymphocytes, mainly of the $CD4^{+}$ type (Figure 4g).[87-90] The application of anti-cytokeratin antibodies allows easy identification of LELs, which appear as negative images (Figure 4d).[33,84] In case of blastic transformation, the Ki-67 index is increased; overexpression of p53 and/or c-myc may also be seen.[99,100] Molecular studies show regular clonal rearrangements of Ig-encoding genes: in particular, the occurrence of numerous somatic mutations and the possible detection of ongoing mutations assign MZL to the group of post-germinal center cell derived lymphomas.[101-103] Peng *et al*. have reported the occurrence of a replication error (RER) phenotype in 50% of cases, which might facilitate the onset of tumor.[100] However, this finding has not been confirmed by others, who found no RER phenotype in gastric MALT lymphomas.[104-106] While t(11;14) and t(14;18) are absent (with only a few exceptions, which are matter of debate), there is a series of recurrent aberrations which are thought to play a role in process of lymphomagenesis and include t(1;14)(p22;q32), trisomy 3, and t(11;18) (q21;q21).[107-118] In particular, t(1;14) causes overexpression of bcl-10, a novel apoptotic signaling gene that encodes an amino-terminal caspase recruitment domain (CARD), homologous to that found in several apoptotic molecules.[107,108] Wild-type bcl-10 activates NF-kappaB but induces apoptosis, as shown in MCF7 or 293 cells. Bcl-10 expressed by lymphoma cells carrying t(1;14) exhibits a frame shift mutation resulting in truncation either in or carboxyl terminal to CARD, which activates NF-kappaB, but does not induce apoptosis.[107,108] Mutant bcl-10 overexpression might have a two-fold lymphomagenic function: loss of bcl-10 pro-apoptotic effect may confer a survival advantage to MALT B-cells, and constitutive NF-kappaB activation may provide both anti-apoptotic and proliferative signals mediated via its transcription factors.[107,108] Therefore, great emphasis was given to t(1;14) and bcl-10 overexpression as major events in the development of MALT lymphoma. Recent studies, however, suggest that bcl-10 mutations occur in a smaller number of gastric MZLs than originally thought, although they seem strictly related to a more aggressive clinical course and unresponsiveness to antibiotic treatment.[109-111] The translocation (11;18) is detected in about half gastric MALT lymphomas, while it is absent from nodal and splenic MZLs: it causes the expression of a chimeric transcript fusing 5' API2 on chromosome 11 to 3' MTL on chromosome 18.[116-118] Finally, p53 and/or c-myc mutations would correspond to the final phase of blastic transformation.[99-101]

Among MZLs, the gastric forms have gained special interest both at the clinical and pathologic level, because of their clear-cut pathogenetic correlations with HP infection.[94,95,119] In particular, the infective agent causes the development of acquired MALT within the stomach and sustains a state of chronic inflammation, which shows varying degrees of activity – expressed by the amount of granulocytes – and persists until HP eradication.[120] The prolonged antigenic stimulation facilitates the outgrowth of clones, which produce autoantibodies against structural components of the gastric mucosa and contribute to the maintenance of lymphoid proliferation.[121-130] Within this context, granulocytes are responsible for oxidative phenomena, which cause DNA instability/damage and might herald the appearance of clones with RER phenotype.[90,99,100] The occurrence of chromosomal alterations leads to the selection of more resistant clones (oligoclonal phase), one of which gains advantage over the others and produces an overt small cell MZL (monoclonal phase).[90] At this time, however, the tumor persists only in the presence of HP infection and by the co-operation of $CD4^{+}$ T-cells via a CD40/CD40L mechanism.[122] Furthermore, it tends to remain localized at the primary site for a long time – with possible local diffusion to the regional nodes – because of the peculiar circulation pathway of MALT elements, controlled by the adhesion molecule MAdCAM-1 and homing receptor α4b7.[126] Further late genomic alterations can make the growth independent of the micro-environment

and p53 and/or c-myc mutations can finally cause its transformation into a DLBCL.[90] Based on this model, it becomes understandable why: a) HP eradication produces tumor regression in about 70% of cases (i.e. in cases which still depend on local antigenic stimulation and have appropriate histologic grade and stage[90,125,126]) and b) untreated gastric MZL needs several years to transform into a DLBCL.[90,98] The time interval between HP eradication and lymphoma regression is highly variable (from 4 weeks to 14 months)[90,127,128] and the histologic regression is usually associated with phenomena of sclerosis and hyalinosis of the mucosa. Prolonged follow-up studies have revealed that most patients who have experienced tumor regression remain in complete remission some years following the completion of the antibiotic treatment.[128] Histology seems to represent the best indicator for judging the achievement and maintenance of complete remission, since it has been shown that polymerase chain reaction (PCR) may display a clonal band even 2 years following therapy, which does not predict relapse of the disease and will disappear in the long run.[129] All these factors are relevant for the therapeutic strategy which, at least initially, can be rather conservative, as well as for patients' management, implying indefinite follow-up of the apparently cured cases. Patients who do not obtain tumor regression or display aggressive histology or disseminated disease should be treated according to conventional strategies, which include total gastrectomy and/or chemotherapy, depending on individual risk factors (degree of stomach wall infiltration, systemic diffusion, etc.).[33,90,130]

Nodal marginal zone B-cell lymphoma (± monocytoid B-cells)

Since the cytological, architectural and phenotypic features of the nodal variety do not differ from those of the extranodal form, the differential diagnosis must be made by the exclusion of an evident MALT lymphoma in any of its characteristic sites.[18,19] The nodal form appears to have a higher rate of early relapse than the other marginal zone B-cell lymphomas and an overall survival similar to that of FCL.[20]

Splenic marginal zone B-cell lymphoma (± villous lymphocytes)

Splenic marginal zone lymphoma (SMZL) was included in the REAL Classification as a provisional entity, since the authors felt that further studies were needed to shed light on its histogenesis and in particular whether it was derived from the marginal zone alone or tended to reproduce all the B-cell maturation steps physiologically occurring in the white pulp of the spleen.[18] Although it is now quoted as an accepted entity in the WHO scheme,[19] its histogenesis remains controversial. Several immunologic and molecular data suggest in fact that the tumor may be unrelated to splenic marginal zone B-cells.[131,132]

SMZL has rather different features from those of the two varieties of B-cell marginal zone lymphoma quoted above.[18] In particular, it most often displays: a) dissemination, with intrasinusoidal bone marrow infiltration (Figures 5a and 5b);[133] b) presence of a leukemic component (with a "villous" appearance in about 50% of cases) (Figure 5c);[134] c) splenic involvement, both ring-like around Malpighian follicles and plurifocally in the red pulp (Figure 5d);[135] d) a very indolent clinical course and favorable response to splenectomy.[136,137] In particular, at microscopic examination, the tumor typically shows dimorphic cytology with an inner core of small cells and a peripheral rim of medium-sized clear elements, with a few blasts intermingled (Figure 5e). Features of plasmacellular differentiation may occasionally be seen. These findings along with the phenotypic profile (B-cell marker^{+}, CD5^{-}, CD10^{-}, CD23^{-}, bcl-2^{+weak}, bcl-6^{-}, BSAP^{+}, IRF4^{-}, IgM^{+}, IgD^{+}, DBA.44$^{+/-}$, and CD68$^{+/-}$) (Figures 5b and 5f) allows the distinction of SMZL from HCL (which primarily involves the red pulp) as well as from other B-cell lymphomas (such as B-CLL, LPL, MCL and FCL), which may involve the spleen also producing a marginal zone pattern and imply different therapeutic strategies.[18,49-53,135] On molecular grounds, neoplastic cells display IgV(H) gene mutations, consistent with a post-germinal center cell derivation.[132] In some cases, ongoing mutations have been observed in elements obtained from the spleen: this finding apparently contrasts with a study showing that blood-borne tumor cells from patients with circulating villous lymphocytes do not show signs of ongoing mutations.[132] However, it is possible that ongoing mutations are acquired in the splenic microenvironment. No examples of SMZL showing the t(11;14) and t(14;18) translocations have been described in the literature; by contrast, recurrent abnormalities of chromosomes 1, 3, 7 and 8 are detected in more than half the cases.[131] Finally, a recent report has proposed the existence of an aggressive variant of SMZL, characterized by an increased number of blasts and frequent 7q loss and/or p53 inactivation: this variant should require a different clinical management of the patients.[138]

Follicular lymphoma

This category – termed *follicular center lymphoma* in the REAL Classification[18] and *follicular lym-*

phoma in the WHO scheme[19] – comprises both the centroblastic/centrocytic and centroblastic follicular forms of the UKC.[9,10] Their inclusion as a single group is justified by their shared histogenesis (from follicular center cells), phenotype and chromosomal abnormalities.[18] Follicular center lymphoma (FCL) is usually characterized by the formation of neoplastic follicles (Figure 6a), which – in the lymph node – affect the cortex, paracortex and medulla.[18] Conversely to normal follicles, neoplastic follicles are quite homogeneous in size and shape, tend to grow back-to-back (compressing the interfollicular areas) and lack well-developed mantles.[7,10,18] In less than 5% of cases, the tumor is purely diffuse: the presence of diffuse areas should always be reported and quantified because of its impact on prognosis.[18] On cytological grounds, FCL consists of centrocytes and centroblasts in different proportions (Figures 6a and 6b), which are randomly distributed within the follicles without the typical zoning pattern observed in normal germinal centers and corresponding to clonal maturation and selection.[7,10,18] In view of the variable ratios of centroblasts and centrocytes and, as a consequence, of the different clinical behavior (grade III FCL will be included among aggressive lymphomas) a grading system has been proposed, according to the Berard cell-counting method [grade I: 0-5 centroblasts/high power field (hpf); grade II: 6-15 centroblasts/hpf; grade III: > 15 centroblasts/hpf].[18,19,139] Some centrocytes are always comprised within the interfollicular areas, a finding never observed under physiologic conditions. Occasionally, centrocytes acquire a signet-ring-like appearance (Figure 6c), possibly engendering a misdiagnosis of metastatic adenocarcinoma.[140] In other instances, the tumor exhibits plasmacellular or marginal zone differentiation at the periphery of neoplastic follicles and/or in the interfollicular areas: these findings compel the differential diagnosis vs. extramedullary plasmacytoma and marginal zone lymphoma.[141,142] Phenomena of sclerosis (more frequent in retroperitoneal neoplasms) and necrosis may be seen.[143,144] In particular, in the presence of a fully necrotic node the pathologist should examine silver impregnated slides carefully, since this stain can reveal an otherwise undetectable follicular pattern.[7,10] The latter is quite characteristic of a lymph node involved by FCL, which has undergone massive infarction because of blood vessel infiltration. At phenotypic analysis, lymphomatous cells carry B-cell markers, along with CD10 and bcl-6 gene product, i.e. molecules regularly found in normal follicular center elements.[18,49,68,145-147] CD20 is strongly expressed, while the staining for CD79a is weak-moderate (Figures 6d and 6e). FCL is regularly negative for CD5, DBA.44 and CD68.[18] Monotypic surface and cytoplasmic Ig can be detected only in a minority of cases, even applying the more sensitive antigen retrieval techniques. On the other hand, in more than 95% of cases neoplastic follicles carry the bcl-2 gene product (Figure 6f): this finding, which is related to the occurrence of t(14;18) or bcl-2 gene rearrangements, is of practical relevance, as it causes protection of neoplastic cells against apoptosis and – along with the above mentioned morphologic criteria – contributes to the distinction of FCL from florid follicular hyperplasia.[18,148-152] The latter is in fact characterized by bcl-2 negativity, since the clonal selection occurring within the follicles produces elimination of unsorted follicular center cells via apoptosis. The amount of lymphomatous elements expressing the proliferation-associated nuclear antigen Ki-67 varies from case to case and even from one follicle to the other within the same case: on the whole, the higher the number of centroblasts, the higher the content of Ki-67$^+$ cells (Figure 6g).[153] In general, FCL is characterized by a low proliferative capacity and a strong protection against apoptosis, a combination which justifies on the one hand the resistance of the tumor to conventional chemotherapies, and on the other the application of novel strategies, such as anti-CD20 antibodies and vaccines.[45] As to other biological markers, FCL is negative at the determination of IRF4, with the exception of rare cases with plasmacellular differentiation, which show positivity limited to the plasma cell component (Figures 6h and 6i).[50] BSAP is strongly expressed by neoplastic cells: the detection of this molecule, along with bcl-6 and CD10, allows easy identification of interfollicular tumoral components, thus contributing to the diagnostic process (Figure 6j).[51] Finally, like normal centroblasts and centrocytes, lymphomatous elements are accompanied by follicular dendritic cells: these are observed in variable amounts and can be easily detected by the application of the following markers: CD21, CD23, CD35, and R4/23.[18] From 80% to 90% of FCLs cases carry the t(14;18)(q32;q21) translocation, which involves the bcl-2 gene.[149-152] The translocation is not exclusive to FCL, since it also occurs in a proportion of DLBCLs (see Part II). In particular, it joins the bcl-2 gene at its 3'-untranslated region to IgH sequences, resulting in deregulation of bcl-2 expression. In about 70% of cases the breakpoints on chromosome 18 are clustered within a major breakpoint region (MBR), while in the remaining ones they occur in the more distant minor cluster region (mcr). The role of bcl-2 deregulation in the development of FCL is matter of debate. It is likely that bcl-2 activation is

not enough for the onset of the tumor, which should require the occurrence of other genetic lesions or host factors, such as chronic antigen stimulation and selection. Deletion of chromosome 6 is seen in approximately 20% of cases.[154] Accumulation of p53 mutations, rearrangements of c-myc or inactivation of p16 are frequently observed in case of progression to a DLBCL.[155,156]

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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. The authors thank Ms. Federica Sandri and Mr. Luigi Chilli for their skillful technical assistance.

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