Identification of B-cell lymphoma

A complex group of tumours, B-cell lymphoma accounts for some 5% of cancer cases, with different types arising along the B-lymphocyte differentiation pathway, making classification and diagnosis difficult. Development of immunohistochemistry and a wide variety of antibodies now permits more accurate diagnosis, which is essential for patient management. Phillippa Cheshire AIBMS reviews the current situation, including application of polymerase chain reaction techniques and identification of appropriate genetic markers that provide valuable insight into the chromosomal translocations associated with different forms of the disease.

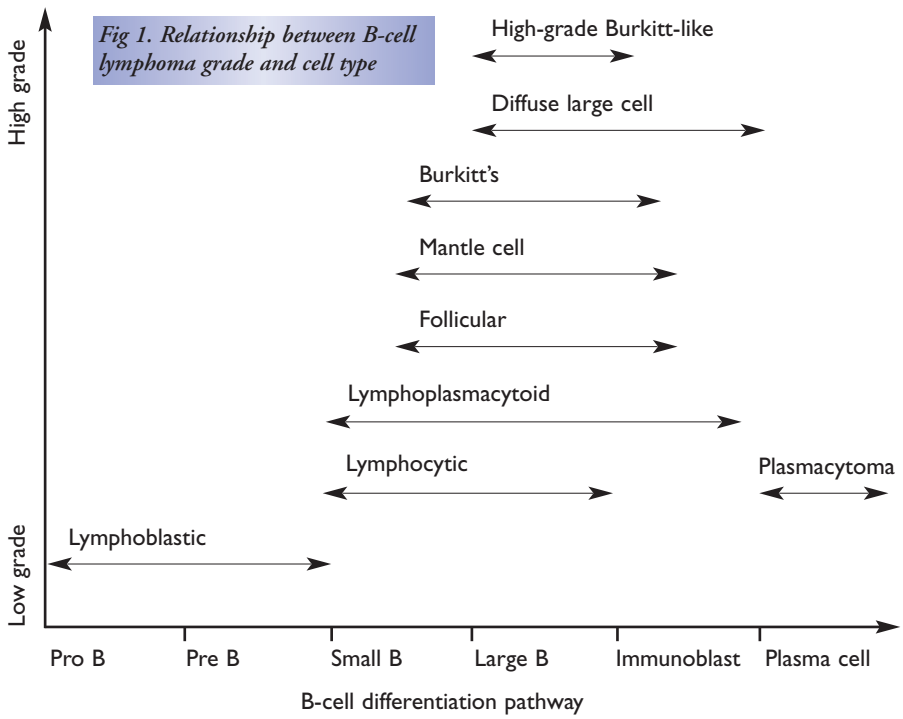
Malignant lymphoma is a cohesive tumourous lesion composed mainly of lymphocytes (and rarely histiocytes) that arises in lymphoid tissue anywhere in the body but most commonly within lymph nodes.[1] Most prominent of these are the B-cell lymphomas, the diagnosis of which can be problematic. Accurate assessment is essential as different prognoses and treatment options are associated with the various types.

Lymphoma classification

Lymphomas are subdivided into two main types: Hodgkin's disease (characterised by Reed-Sternberg cells) and non-Hodgkin's lymphoma (NHL). Classification of NHL has developed over many years, from that of Rappaport in 1966, based on cytological appearances and growth pattern, to the Luke-Collins (1973/4) system and the working formulation that attempted to relate different lymphomas to developmental stages of the normal immune system; however, these did not take into account fully the different cell types, and were clinically orientated. Subsequently, the Keil classification subdivided lymphomas into T-, B- and histiocytic cell types but ignored extranodal lymphoma (40% of all cases).[2]

Development of immunohistochemistry has increased understanding of the underlying disease process, and led to the development of immunotherapy and the formation of an international lymphoma study group. The result is the Revised European American Lymphoma (REAL) system of classification, which lists all identifiable lymphomas with distinct clinicopathological patterns, including extranodal lymphoma. Distinct tumours are classified according to cell type and a specific set of parameters (morphology, phenotype, molecular characteristic and clinical aspects). The REAL system is now used in many laboratories throughout the world,[3] and Figure 1 shows the relationship between B-cell lymphoma differentiation and grade.

Fig 1. Relationship between B-cell lymphoma grade and cell type



Detection and diagnosis

Generally, lymphomas are monoclonal in origin and can spread easily, usually to the spleen, liver and bone marrow. They can go unnoticed for some time but ultimately produce local or generalised lymphadenopathy, systemic symptoms or those related to the particular organ involved. At diagnosis almost a third of patients will have metastatic disease.[1,2] Initially, blood counts may show autoimmune haemolytic anaemia and thrombocytopenia, but more commonly low serum immunoglobulin levels.[4]

Diagnosis by fine-needle aspiration (FNA) is difficult and largely based on the presence of an almost completely monomorphous malignant cell type. In comparison, reactive conditions normally show a varied cell population. Cells with large prominent nuclei (indicative of malignancy at other sites) may indicate only benign change or lymphoid cell differentiation,[5] and lymphomas that arise at different stages of cell development further complicate diagnosis. Accurate diagnosis requires tissue biopsy and histological examination of the

Table 1. B-cell lymphoma antibody expression

Lymphoma type	Pan B	sIg	cIg	CD5	CD10	CD23	CD34	CD43	Other
Pre B cell	4	0	0	0	3	0	3	0	TdT CD22 CD19
Lymphocytic	4	D3	2	4	1	4	0	4	CD37
Lymphoplasmacytoid	4	M4 D2	4	1	1	0	0	3	
Mantle cell	4	M&D 4	0	4	1	1	0	4	L>K cyclin-D1+ve
Follicular	4	4	0	1	3	2	0	1	*Bcl*-2+ve
Extranodal marginal zone	4	M4	2	1	1	1	0	2	
Nodal marginal zone	4	M4 D1	2	1	1	1	0	2	
Splenic marginal zone	4	M4	2	1	1	1	0	0	CD25-ve
Hairy cell	4	4	0	0	0	0	0	2	CD11c CD25, CD103 DBA 44
Plasmacytoma	1	0	4	0	0	0	0	0	CD38+ve
Diffuse large cell	4	3	2	2	2	0	0	1	*Bcl*-2 in 30% CD79a
Burkitt's	4	M4	0	1	4	0	0	0	EBV+ve
High grade	4	3	2	1	2	0	0	0	

Footnote: 0 = negative, 1 = <10% positive, 2 10-50% positivity, 3 = 50-90% positivity and 4 = >90% positive

tissue architecture and cell morphology, and the extent of disease is determined by abdominal ultrasound, lymphangiography and bone-marrow examination.

A range of monoclonal antibodies is now available for use on formalin-fixed, paraffin-embedded tissues; however, there remain some that will only react with fresh tissue. Decalcification is another histological process that can affect antigens present in tissue, and this is of particular relevance to bone-marrow trephine specimens. This notwithstanding, monoclonal antibodies are used extensively to determine the precise cell lineage, particularly in cases where the available morphological information is inconclusive. The initial panel used includes antibodies to leucocyte common antigen, CD20 (B-cell marker), CD3 (T-cell marker) and UCLH1 (positive with T-cell lymphoma and immunoblastic B-cell lymphoma).[6]

Many monoclonal B-cell antibodies are available, each of which is given a cluster differentiation (CD) number that indicates the specific antigen against which it was raised. In particular, 12 are specific for B cells (or occur at low incidence on other cells) and are useful diagnostically (Table 1).[7]

Many malignant lymphomas will show one or more immunophenotypic abnormalities, and demonstrate either kappa or lambda positivity, but not both. Thus, so-called light-chain restriction and the presence of CD5 positivity can be used to distinguish malignant conditions.[8] Another example of immunophenotypic abnormality is the expression of CD43, a T-cell marker that occurs in 60% of mantle-cell lymphomas, 39% of small lymphocytic lymphomas, 16% of diffuse large-cell lymphomas and 5% of follicular lymphomas.[9,10]

Genetic analysis is limited to specialist centres and is largely research-based; however, chromosomal translocations associated with B-cell lymphoma can be detected in cytogenetics laboratories. Of particular significance is the *Bcl*-2 gene rearrangement seen in follicular lymphoma. Such translocations often occur during normal cell differentiation, when progenitor B cells mature to pre-B cells and the heavy-chain variable region is assembled, during light-chain rearrangement at the pre-B-cell stage, or during differentiation into plasma and memory cells when heavy-chain class switching occurs. Translocation often involves oncogenes and tumour suppressor genes but the precise role of many remains unclear.[11]

Types of B-cell lymphoma

The following are a selection of the more interesting B-cell lymphoma types that may be encountered. A more comprehensive list, including antigen and genetic characteristics, may be found in Table 1.

Lymphoblastic lymphoma

Precursor B-cell lymphoblastic lymphoma is an aggressive lymphoma that occurs in children and affects the skin and bone marrow. Histology shows loss of lymph node architecture and capsular destruction. Cells are generally of small to medium size with inconspicuous nuclei that show a fine chromatin pattern and high mitotic rate. It can be confused with other NHL and therefore immunohistochemical analysis is essential, with TdT, CD19 and CD22 being of particular use. Heavy-chain gene rearrangements very often can be identified but only 40% show light-chain translocation. It is treatable by multi-agent chemotherapy.[2,7]

Lymphoplasmacytoid lymphoma

Also called lymphoplasmacytic immunocytoma, this is a low-grade lymphoma of small lymphocytes, plasma cells and intermediate forms. An uncommon type, it occurs in the elderly, who show signs of Waldenstrom's macroglobulinaemia. Distinctive features include the presence of Outcher and Russell bodies, although in difficult cases immunohistochemistry is essential. Neoplastic cells are positive for surface IgM antibody but negative for CD5 and CD10. The tumour shows both heavy and light chain gene rearrangements, the most frequent being t(9; 14)(p13; q32), which relates to the *PAX5* gene locus. This encodes a transcription ➤

Mantle-cell lymphoma

Although classed as a low-grade lymphoma, this form occurs in older adults and has a poor prognosis. At diagnosis the disease very often has spread throughout the body, with deposits in the bone marrow, spleen and gastrointestinal tract (GIT), where it presents as a polypoid mass. Three variants are seen and include classical, blastoid and pleomorphic forms. In classical mantle-cell lymphoma, a neoplastic infiltrate replaces the mantle zone (Figures 2a and 2b), leaving naked germinal centres that also may be replaced by lymphoma cells. Cells are small to medium sized with irregular nuclei showing condensed chromatin and scanty cytoplasm. In the blastoid form, cells resemble lymphoblasts with very fine chromatin, little cytoplasm and a high mitotic rate. The pleomorphic variant has focally larger, more hyperchromatic neoplastic cells that show nuclear clefts. They are positive for surface IgM and IgG, CD43 (60%) and CD5 (80 %), and are distinguished from B-cell lymphocytic lymphoma by the absence of CD10 and CD23. A third show a t(11; 14) (q13; q32) chromosome rearrangement, which results in over-expression of the *bcl*-1 gene and consequent production of cyclin-D1 protein (Figure 2c). These alterations are of clinical significance as new antibodies are produced for routine diagnosis and primers developed to allow detection of the translocation by the polymerase chain reaction (PCR).[2,13]

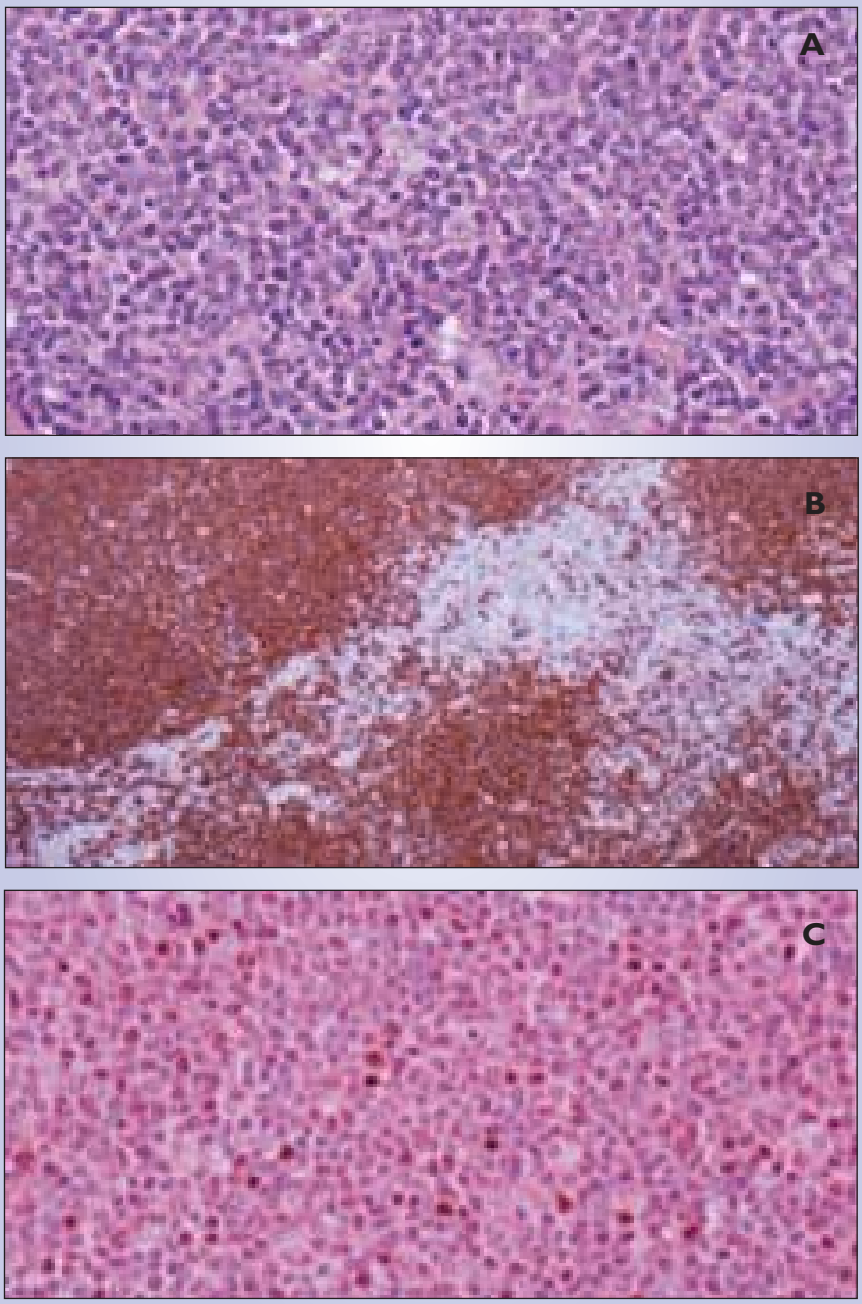


Fig 2. Histological appearance of mantle-cell lymphoma: haematoxylin and eosin stain (a) and immunohistochemistry showing CD20 (b) and cyclin-D1 (c) positivity.

factor protein involved in the B-cell differentiation pathway and the translocation is thought to result in down-regulation of the protein. This form is incurable with current therapies.[2,4,11,12]

Marginal zone lymphoma

This can be divided into two types: mucosa-associated lymphoid tissue (MALT) lymphoma, and nodal marginal zone B-cell (or monocytoid) lymphoma. Both express surface immunoglobulin, with half expressing core immunoglobulin and co-expressing CD43 as well as the normal B-cell markers. CD5, 10 and 23 are usually negative. Chromosomal translocations involving the *API2*, *MLT* and *bcl*-10 genes have been identified.

MALT lymphoma affects extranodal mucosal and epithelial tissues, especially the GIT, salivary glands, lung and thyroid. It is a low-grade lesion curable by surgery or radiotherapy, but some cases progress to diffuse large B-cell lymphoma. Patients often have a history of autoimmune disease or antigenic stimulation. The tumour consists of small lymphocytes, marginal zone cells and/or monocytoid B cells with large lymphoblastic cells, and the infiltrate involves the perifollicular, interfollicular or follicular areas of the node. Trisomy 3 is common and some patients show chromosome 12 and 18 abnormalities, although no pathologically significant translocation has yet been identified.[2,7,14]

Monocytoid B-cell lymphoma affects adults and approximately 40% will have bone marrow involvement and 10% go on to develop into large-cell lymphoma. The monocytoid cells are small to medium in size with an irregular nuclear outline, condensed chromatin and moderately abundant clear to pale cytoplasm. There are scattered immunoblasts, some of which have Dutcher bodies, and mature plasma cells. The lymphoma develops from a *bcl*-1 and -2 germ line.[2,7]

Splenic marginal zone lymphoma affects adults. It presents with splenomegaly and mild to moderate lymphocytosis, at which stage it is usually high-grade and incurable. The cells have larger nuclei with less condensed chromatin than that found in other marginal zone lymphomas. Genetic alterations are virtually unknown, although some tumours exhibit *p53* gene rearrangement.[2,7,15]

Hairy cell lymphoma

Adults who have hairy cell leukemia (a late-stage B-cell lymphoma) present with an enlarged spleen that shows blood-lake formation, and cytopenia with increased episodes of infection. The neoplastic cells are small to medium in size and have plentiful pale cytoplasm with long thin surface projections seen on smears. The nuclei may have a smooth or indented nucleus. The tumour expresses surface immunoglobulin, CD11c, CD25,

CD103, DBA44, and is positive with B-cell markers. Rearrangement of the immunoglobulin genes and deletion of the *p53* gene is common. The disease is slow to progress and remission can be induced by various drugs, especially interferon-α.[2,7, 15,16]

Diffuse large-cell lymphoma

This is an aggressive type seen across a wide age range. It is potentially curable, with two-thirds of all cases present at a single site and a 60% survival rate following multidrug chemotherapy. The node shows a diffuse architecture, and a significant number of the neoplastic cells are large and either cleaved, non-cleaved, multilobulated or immunoblasts and have abundant pale cytoplasm. Some may have a population of small neoplastic cells, and sclerosis is seen in half of all cases. Generally, B-cell markers are positive, especially CD20 and CD79a, and there is co-expression of CD43 and CD5. Heavy and light chain immunoglobulin gene rearrangements have been identified, particularly *bcl*-2 (20 %) and *bcl-6/LAZ3* genes, but this has neither diagnostic nor prognostic significance due to the heterogeneity of this lymphoma. Considerable cellular variation is seen and there has been much debate about the need for categorisation into specific cell types (ie immunoblastic; diffuse centroblastic and centroblastic polymorphic or mixed small and large cell; diffuse large cell and large immunoblastic). This arises from the variation in lymphoma classification used by Kiel where clinical information had greater emphasis than pathological data. One subtype readily identifiable is the primary mediastinal large B-cell lymphoma. This occurs in young adults, mainly females, and is composed of distinctive large neoplastic cells.[2,7,17,18]

Burkitt's lymphoma

The endemic form occurs in Africa and affects mainly children (more commonly boys) who present with jaw tumours and other extranodal masses. It is aggressive but curable with multidrug chemotherapy and possibly bone-marrow transplantation, but this is being investigated currently. Microscopic appearance shows a monotonous cell population of medium-sized cells with round nuclei showing a vesicular chromatin pattern, and a rim of basophilic cytoplasm that tends to mould against other cells. Interspersed macrophages produce the 'starry sky' pattern that is characteristic of the disease. Cells express most B-cell markers as well as surface IgM and CD10. Nearly all cases are associated with the presence of Epstein-Barr virus (EBV) and show rearrangement of the *c-myc* gene on chromosome 8 (t[8; 14] [q24.1; q23.3]), although its precise role is unclear.[2,4,7,11]

Alterations to classification

The REAL system was reviewed by the World Health Organisation (WHO) in 1997 and a few alterations to classification were made, the most notable of which being the renaming of immunocytoma as lymphoplasmacytic lymphoma. Nodal and splenic marginal zone lymphomas became accepted categories, and the Burkitt-like lymphoma type absorbed into large B-cell lymphoma.[3]

In summary

Advances in genetic testing will allow gene rearrangements to act as more sensitive markers of disease (PCR replacing Southern blot). Recently PCR amplification of the FR3 region of the immunoglobulin heavy gene has been used to distinguish benign cases from malignant low-grade B-cell lymphoma on both frozen and formalin-fixed, paraffin-embedded tissue, and a success rate of approximately 62% achieved. The success rate is low in follicular lymphomas as the translocation of the *bcl*-2 gene is thought to result in the loss of the primer binding site, although this alteration has become of diagnostic and prognostic significance. Identification of *p53* gene rearrangement in lymphoma is thought to indicate advanced disease and poor prognosis.

Such examples indicate areas where genetic analysis can provide an adjunctive test to routine histological diagnosis, especially as the test can be carried out within 24 hours on small amounts of tissue or blood.[19] Future development will see genetic analysis become more cost effective and user friendly, and this will help not only to classify B-cell lymphomas more accurately but also give greater insight into the disease process and new treatment options. ■

References

1 Cotran, Kumar, Robbins, eds. *Robbins pathological basis of disease,* 4th ed. Saunders, 1989.

2 Chan JKC, Banks PM, Cleary ML, *et al.* A proposal for classification of lymphoid neoplasms (by the international lymphoma study group). *Histopathology* 1994; **25**: 517-36.

3 Pileri SA, Milani M, Fraternali-Orcioni G, Sabattini E. From the REAL classification to the upcoming WHO scheme: a step towards universal catagorisation of lymphoma entities? *Annal Oncol* 1998; **9**; 607-12.

4 Chapel, Haeney, eds. *Essentials of clinical immunology*, 3rd ed. Blackwell Science, 1993.

5 Leong ASY, Stevens M. Fine needle aspiration biopsy for the diagnosis of lymphoma: a perspective. *Diagn Cytopathol* 1996; **15**: 352-7.

6 Jasani B, Schmid KW. *Immunohistochemistry in diagnostic histopathology*. Churchill Livingstone, 1993.

7 Stevenson GT, Cragg MS. Molecular markers of B cell lymphoma. *Semin Cancer Biol* 1999; **9**: 139-47.

8 Picker LJ, Wiess LM, Medeiros LJ, Wood GS, Warnke RA. Immunophenotypic criteria for the diagnosis of non-Hodgkin's lymphoma. *Am J Pathol* 1987; **128**: 181-201.

9 Gelb AB, Rouse RV, Dorfman RF, Wanke RA. Detection of immunophenotypic abnormalities in paraffin embedded B lineage non-Hodgkin's lymphomas. *Am J Clin Pathol* 1994; **102(6)**: 825-34.

10 Kurtin PJ, Hobday KS, Ziesmer S, Caron BL. Demonstration of distinct antigenic profiles of small B cell lymphomas by paraffin section immunohistochemistry. *Am J Clin Pathol* 1999; **112(3)**: 319-29.

11 Hickish T, Cunningham D. Gene rearrangements in B cell lymphomas. In: Carney D, Sikora K, eds. *Genes and cancer.* John Wiley & Sons, 1990.

12 Ohno H, Ueda C, Akasks T. The t(9; 14)(q13; q32) translocation in B cell non-Hodgkin's lymphoma. *Leuk Lymphoma* 2000; **36**: 435-45.

13 Hankin RC, Hunter SV. Mantle cell lymphoma. *Arch Pathol Lab Med* 1999; **123**: 1182-8.

14 Dierlamm J, Wlodarska I, Michaux L, *et al.* Genetic abnormalities in marginal zone B cell lymphoma. *Hematol Oncol* 2000; **18**: 1-13.

15 Chen PM, Chiou TJ, Hsieh RK, *et al.* p53 gene mutations and rearrangements in non-Hodgkin's lymphoma. *Cancer* 1999; **85**: 718-24.

16 Vallianatou K, Brito-Babapulle V, Matutes E, *et al.* p53 gene deletion and trisomy 12 in hairy cell leukemia and its variants. Leuk Res 1999; **23**: 1041-5.

17 Hosokawa Y, Maede Y, Ichinohasama R, *et al.* The Ikaros gene, a central regulator of lymphoid differentiation, fuses to the BCL 6 gene as a result of t(3; 7)(q27; p12) translocation in patients with diffuse large B cell lymphoma. *Blood* 2000; **95**: 2719-21.

18 Lossos IC, Okada CY, Tibshirani R, *et al.* Molecular analysis of immunoglobulin genes in diffuse large B cell lymphomas. *Blood* 2000; **95**: 1797-803.

19 Pittaluga S, Tierens A, Dodoo YL, *et al.* How reliable is histological examination of bone marrow trephine biopsy specimens for the staging of non-Hodgkin's lymphoma? A study of hairy cell leukemia and mantle cell lymphoma involvement of bone marrow trephine specimens by histologic, immunohistochemical and polymerase chain reaction techniques. *Am J Clin Pathol* 1999; **111**: 179-84.

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