Identification of B-cell lymphoma

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. 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).

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, and Figure 1 shows the relationship between B-cell lymphoma differentiation and grade.

**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.

Initially, blood counts may show autoimmune haemolytic anaemia and thrombocytopenia, but more commonly low serum immunoglobulin levels.

Diagnosis by fine-needle aspiration (FNA) is difficult and largely based on the presence of an almost completely monomorphic 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, and lymphomas that arise at different stages of cell development further complicate diagnosis. Accurate diagnosis requires tissue biopsy and histological examination of the

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

- High-grade Burkitt-like
- Diffuse large cell
- Burkitt’s
- Mantle cell
- Follicular
- Lymphoplasmacytoid
- Lymphocytic
- Plasmacytoma

**B-cell differentiation pathway**

- Pro B
- Pre B
- Small B
- Large B
- Immunoblast
- Plasma cell
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. 12 are specific for B cells (or occur at low incidence on other cells) and are useful diagnostically (Table 1).7

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Table 1. B-cell lymphoma antibody expression

<table>
<thead>
<tr>
<th>Lymphoma type</th>
<th>Pan B</th>
<th>slg</th>
<th>clg</th>
<th>CD5</th>
<th>CD10</th>
<th>CD23</th>
<th>CD34</th>
<th>CD43</th>
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<td>D2</td>
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Footnote: 0 = negative, 1 = <10% positive, 2 = 10-50% positivity, 3 = 50-90% positivity and 4 = >90% positive

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 Dutcher 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 factor specific to plasma cells.
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.\textsuperscript{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 $\texttt{API2}$, $\texttt{MLT}$ and $\texttt{bcl}$-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.\textsuperscript{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 $\texttt{bcl}$-1 and -2 germ line.\textsuperscript{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.\textsuperscript{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,

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**Fig 2.** Histological appearance of mantle-cell lymphoma: haematoxylin and eosin stain (a) and immunohistochemistry showing CD20 (b) and cyclin-D1 (c) positivity.
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 neoplastic cells have 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/LA23 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 specific cell types (ie immunoblastic; seen and there has been much debate due to the heterogeneity of this lymphoma). Diffuse centroblastic lymphoma and the Burkitt-like lymphoma types absorbed into large B-cell lymphoma.2

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.

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. 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

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 immunocyto- toma as lymphoplasmacytic lymphoma. Nodal and splenic marginal zone lymphomas became accepted categories, and the Burkitt-like lymphoma type absorbed into large B-cell lymphoma.2

References