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Lymphoma diagnosis: an update

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Malignant lymphomas are a heterogeneous group of tumours that arise from cells of the immune system. Over 40 distinct clinicopathological entities are currently recognised under the broad heading of lymphoma, a number that is bound to increase as understanding of disease biology advances. The clinical picture, treatment and prognosis of these disorders may be very different so diagnostic accuracy is of paramount importance. Genetic and phenotypic markers can also predict prognosis and response to treatment within individual disease categories – of increasing relevance given the availability of risk-adjusted and targeted therapies.

This article summarises the current approach to lymphoma diagnosis and indicates how it is likely to evolve in the future.

Lymphoma classification

Advances in basic cellular and molecular biology, combined with the application of new investigative techniques, have transformed our understanding of the lymphomas over the last 25 years. Descriptive classifications based on morphological appearance have given way to the current World Health Organization (WHO) system (Table 1),[1] which integrates information on the cellular origin of the tumour with its genetic and phenotypic features and the clinical picture. This works well for B cell lymphomas,[2] but is at present less satisfactory for the T cell disorders as the normal counterpart of the malignant cell is often unknown.

Clinical presentation

The complexity and ubiquitous nature of the immune system mean that lymphomas can present to almost any specialty with symptoms and signs that depend on the underlying disease biology and the anatomical site(s) involved. Some lymphomas pursue a highly indolent course with few symptoms or signs for many months or years; others progress rapidly, sometimes following an initial low-grade phase. Nodal enlargement is a common presentation but a significant number occur at extranodal sites such as the skin, gastrointestinal tract, lung, thyroid and parotid glands, frequently in association with chronic infection or organ specific autoimmunity.[3]

Diagnosis

The result of a blood count and film should always be available before performing invasive investigations since this may be all that is required to identify conditions such as chronic lymphocytic leukaemia (CLL) and non-malignant disorders like glandular fever. A number of viral infections are associated with lymphoma, including HIV, human T lymphotropic virus-1 and hepatitis C, whilst others such as hepatitis B can reactivate during chemotherapy. Screening for these viruses is therefore recommended.

As a general rule, lymph node enlargement present for six weeks without explanation should be considered suspicious of lymphoma and the patient referred appropriately, although sometimes abnormalities such as large-volume mediastinal lymphadenopathy will demand immediate action. Wherever possible, excision biopsy of the involved lymph node remains the preferred route to diagnosis because it gives information about tissue architecture and ensures the availability of adequate material for more detailed analysis. For some investigations, fresh tissue is preferable to traditional formalin fixation. Robust protocols must be in place to ensure prompt processing.

Lymphadenopathy involving the head and neck should be investigated in conjunction with an ear, nose and throat or faciomaxillary specialist since squamous

cell cancers have a worse prognosis following excision biopsy and should be sampled by fine-needle aspiration cytology in the first instance. Basic cytology is usually inadequate for the diagnosis of lymphoma, but techniques such as multiparameter flow cytometry may in future expand the role of this modality.

Where peripheral tissue is not accessible, needle biopsy guided by computed tomography or ultrasound, mediastinoscopy or laparoscopy may be required. Suspected recurrence should be confirmed by repeat biopsy, as in some cases the lymphoma may be of a different subtype at relapse.

Diagnostic techniques

Morphology and immunophenotyping

At present, morphological assessment of stained peripheral blood or tissue sections is a key component of the diagnostic process and guides the application of further, more sophisticated immunological and molecular genetic techniques. Immunophenotyping involves the use of labelled antibodies to identify surface or intracellular antigens. In tissue sections,

these are usually detected using histochemical colour reactions so that only one or two antigens can be visualised simultaneously. Flow cytometry allows simultaneous expression of multiple antigens to be assessed but this technique is applicable only to suspensions of fresh cells.

Molecular genetics

Molecular genetic techniques are assuming an increasingly important role in the diagnosis of lymphoma and other cancers. In addition to their role in

defining diagnosis and prognosis, these methods may also provide information about disease pathogenesis and normal biology. It is interesting to note that a number of important genes such as the apoptosis regulator BCL2 were discovered in this way.

Conventional metaphase cytogenetics, which describes the morphological appearance of the chromosomes in dividing cells, is now routinely supplemented by techniques such as fluorescence *in situ* hybridisation (FISH) in which labelled sequence-specific DNA probes can identify deletions and

Key Points

Accurate diagnosis of lymphoma is crucial for the delivery of appropriate therapy. Diagnostic samples should be reviewed by an expert haematopathologist

Excision biopsy of the suspect lymph node is preferred, preceded by fine-needle aspirate cytology when head and neck carcinoma is a possibility. Robust protocols should be in place to prevent diagnostic delays

A number of viral infections are associated with lymphoma whilst others may reactivate during therapy. Appropriate virological screening should be performed in all newly diagnosed cases of lymphoma

Response to therapy and prognosis may be predicted by phenotypic and molecular biomarkers

KEY WORDS: diagnosis, lymphoma

Table 1. Summary of World Health Organization (WHO) classification (a simplified version of the WHO classification of mature lymphoid tissue tumours). © Copyright World Health Organization, 2007. All rights reserved.[1]

Non-Hodgkin lymphoma (70% of all lymphoma)		Hodgkin lymphoma (30% of all lymphoma)
Mature B cell neoplasms (90% of all B and T cell neoplasms)	Mature T cell and NK neoplasms (10% of all B and T cell neoplasms)	
Disseminated/leukaemic: – chronic lymphocytic leukaemia (6.7%) – B cell prolymphocytic leukaemia – lymphoplasmacytic lymphoma – splenic marginal zone lymphoma – hairy cell leukaemia	Disseminated/leukaemic: – T cell prolymphocytic leukaemia – T cell large granular lymphocytic leukaemia – adult T cell leukaemia/lymphoma	Nodular lymphocyte predominant Hodgkin lymphoma (5% of all cases of Hodgkin lymphoma) Classical Hodgkin lymphoma: – nodular sclerosis – mixed cellularity – lymphocyte rich – lymphocyte depleted
Nodal and extranodal B cell non-Hodgkin lymphoma: • diffuse large B cell lymphoma (30.6%) • follicular lymphoma (20.1%) • extranodal marginal zone lymphoma (7.6%) • mantle cell lymphoma (6.0%) • Burkitt lymphoma (2.5%) • mediastinal large B cell lymphoma (2.4%) • nodal marginal zone lymphoma (1.8%) • other rarer subtypes	Nodal: – peripheral T cell lymphoma, unspecified – angioimmunoblastic T cell lymphoma – anaplastic large cell lymphoma (2.4%) Cutaneous: – mycosis fungoides/Sezary syndrome	

translocations in both dividing and non-dividing cells in cell suspensions and tissue sections. Many lymphomas are characterised by translocations involving the immunoglobulin heavy chain gene on chromosome 14. For example, in follicular lymphoma, the BCL2 gene, which regulates cell death, is aberrantly expressed because of a translocation between chromosomes 14 and 18.

These lesions may also be detected by the polymerase chain reaction, a highly sensitive amplification technique that can be used to confirm clonality by detecting rearrangements of the B or T cell receptors. Increasingly, gene expression profiling (GEP), in which the mRNA level of all or a subset of expressed genes are measured simultaneously, is being used to reclassify disease subtypes and identify prognostic markers. Molecular methods and their application in the diagnosis of haematological malignancies are reviewed elsewhere.[4]

Integrated approach to diagnosis and prognosis

When used together, the techniques described above are able to provide accurate diagnostic and prognostic information. Examples of the use of this integrated approach in the diagnosis of several of the more common lymphomas are given below. (A comprehensive account of lymphoma diagnosis is provided by the WHO 'blue book'.[1])

Fig 1. Diffuse large B cell lymphoma (DLBL) and Burkitt lymphoma (BL) morphology and immunohistochemical findings: (a) in DLBL a diffuse infiltrate of large transformed cells with prominent central nucleoli has replaced the normal lymph node architecture. The cells express the mature B cell antigen CD20 and have a high (>40%) proliferation fraction, in this case detected by Ki67 staining; **(b)** BL morphology shows monotonous medium sized cells with multiple nucleoli, usually with a 'starry sky' appearance caused by surrounding benign macrophages that have ingested apoptotic tumour cells. BL cells stain for CD20 and nearly 100% express Ki67.

Fig 2. Diffuse large B cell lymphoma (DLBL) and Burkitt lymphoma (BL). BL fluorescence *in situ* hybridisation (FISH) showing the t(8;14) abnormality. Cases of BL have a translocation of the *MYC* gene from band q24 of chromosome 8 (red) to either the immunoglobulin heavy chain (IGH) locus on chromosome 14 (green) or, less frequently, the light chain kappa or lambda regions on chromosomes 2 and 22, respectively. 8cen refers to the centromere of chromosome 8. The t(8;14) fusion product is shown as a yellow signal in the left-hand cell; the right-hand cell is normal.

Aggressive B cell lymphomas

For clinical purposes, the most important distinction to make in the diagnosis of aggressive B cell lymphoma is between diffuse large B cell (DLBL) and Burkitt lymphomas (BL) (Figs 1–3). Although both follow an aggressive course, BL has a very high proliferation rate associated with chromosomal translocations involving the MYC gene and a propensity to spread to the central nervous system (CNS). If treated with appropriately intensive therapy, including CNS prophylaxis, BL can have a very good prognosis. However, recent studies have revealed that, following expert review and molecular analysis, some cases thought to be DLBL are in fact BL and vice versa.[5] DLBL is a heterogeneous disorder within which subtypes with differing prognosis and treatment response have been identified by immunophenotyping[6] and GEP.[7] Germinal centre type cases of DLBL identified by expression of CD10 and bcl-6 have a good prognosis compared with the activated B cell type. Mediastinal large B cell lymphoma has been shown to posses a GEP intermediate between DLBL and Hodgkin lymphoma which also frequently presents with a mediastinal mass.
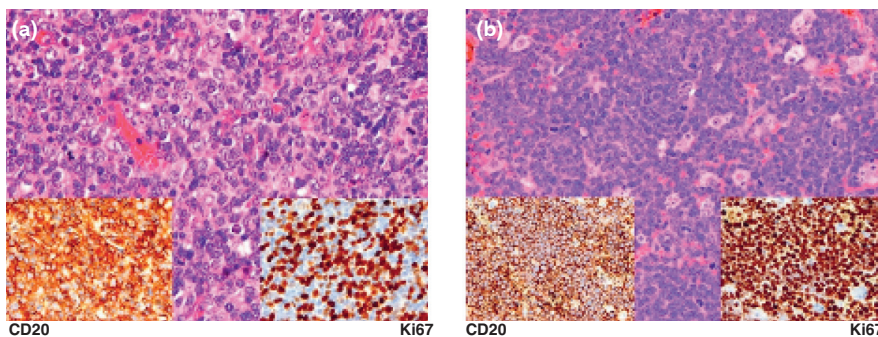
Indolent B cell lymphomas

The slowly progressive, or low-grade lymphomas encompass disorders with a wide spectrum of natural history from the extremely indolent subtypes of CLL, which may never require therapy and have a survival identical to age-matched controls, to follicular and mantle cell lymphomas which have a median survival of 10 and five years, respectively. Many can present with a raised peripheral blood lymphocyte count and are readily confused with each other so that an integrated approach to diagnosis is required (Figs 4–6).
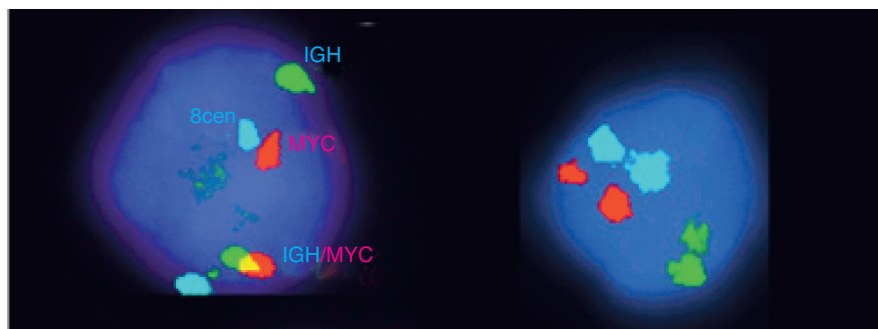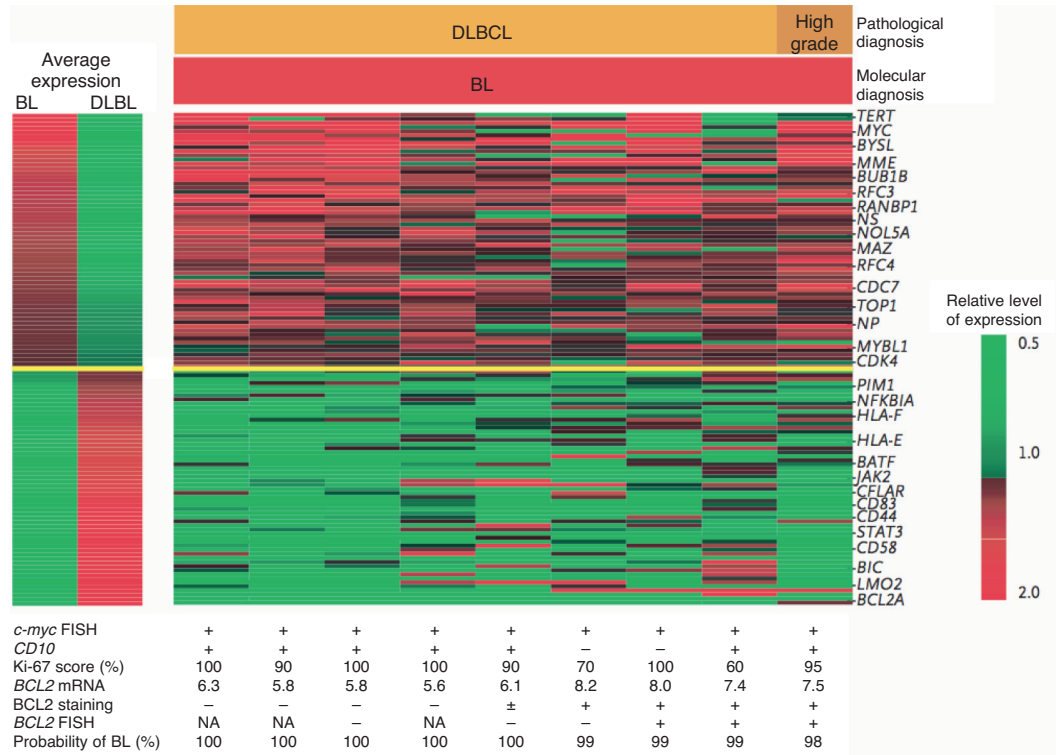
Service organisation

Despite an accurate diagnosis being the essential prerequisite for successful lymphoma treatment, a number of

Fig 3. The potential diagnostic value of gene expression profiling (GEP) in Burkitt lymphoma (BL) and diffuse large B cell lymphoma (DLBL). Nine of the 71 cases in this study originally designated as BL showed a discrepancy between the pathological and molecular diagnosis based on GEP. BL could be distinguished from DLBL by the high level of expression of MYC target genes, the expression of a subgroup of germinal centre B cell genes, and the low level of expression of major histocompatibility complex class I genes and NF-kappa B target genes. FISH = fluorescence *in situ* hybridisation. Copyright © 2007 Massachusetts Medical Society. All rights reserved.[5]

| | | DLBCL | | | | | | High grade | Pathological diagnosis |
| BL | | | | | | | | | Molecular diagnosis |

Genes listed: TERT, MYC, BYSL, MME, BUB1B, RFC3, RANBP1, NS, NOL5A, MAZ, RFC4, CDC7, TOP1, NP, MYBL1, CDK4, PIM1, NFKBIA, HLA-F, HLA-E, BATF, JAK2, CFLAR, CD83, CD44, STAT3, CD58, BIC, LMO2, BCL2A

Relative level of expression: 0.5 (green) – 1.0 – 2.0 (red)

c-myc FISH	+	+	+	+	+	+	+	+	+
CD10	+	+	+	+	+	−	−	+	+
Ki-67 score (%)	100	90	100	100	90	70	100	60	95
BCL2 mRNA	6.3	5.8	5.8	5.6	6.1	8.2	8.0	7.4	7.5
BCL2 staining	−	−	−	−	±	+	+	+	+
BCL2 FISH	NA	NA	−	NA	−	−	+	+	+
Probability of BL (%)	100	100	100	100	100	99	99	99	98

studies have documented worrying inconsistencies in this area. For example, central review of 745 lymph node biopsies performed in Wales in 1998–2000 revealed diagnostic discordance in 17% of cases, of which 36% would have led to a change in management.[8] These and other observations led the National Institute for Health and Clinical Excellence to mandate major service reconfiguration in the 2003 guidance on improving outcomes in haematological cancers.[9] Specific recommendations include:

- review by an expert haematopathologist of all new diagnoses of haematological malignancy
- service rationalisation, so that each cancer network has no more than one immunophenotyping, cytogenetic and molecular diagnostic laboratory
- production of an integrated diagnostic report that combines all the above modalities
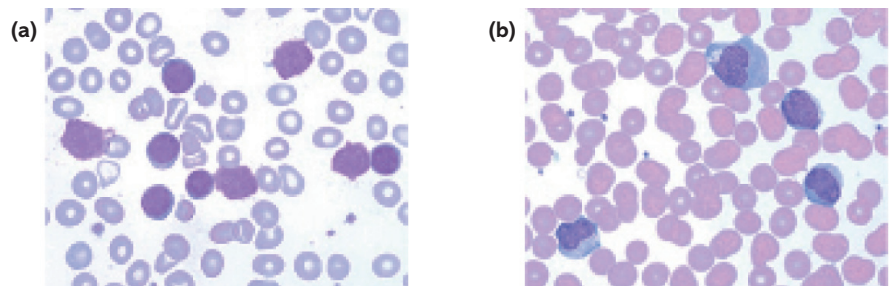- treatment decisions to be made by a multidisciplinary group which

Fig 4. Chronic lymphocytic leukaemia (CLL) and mantle cell lymphoma (MCL) may both present with a peripheral blood lymphocytosis. The blood films show an excess of small mature lymphocytes: **(a)** CLL cells are generally more monotonous and smear cells are seen; **(b)** MCL cells are more heterogeneous and often show indented nuclei. The distinction between these disorders is important because MCL usually follows a more aggressive course than CLL and requires more aggressive therapy.

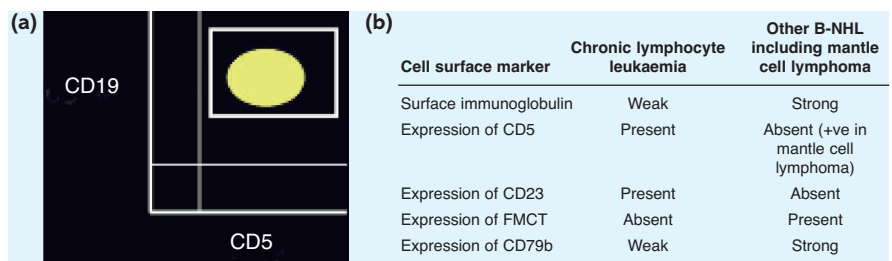| Cell surface marker	Chronic lymphocyte leukaemia	Other B-NHL including mantle cell lymphoma
Surface immunoglobulin	Weak	Strong
Expression of CD5	Present	Absent (+ve in mantle cell lymphoma)
Expression of CD23	Present	Absent
Expression of FMCT	Absent	Present
Expression of CD79b	Weak	Strong

Fig 5. Chronic lymphocytic leukaemia (CLL) and mantle cell lymphoma (MCL). Flow cytometry enables the immunophenotype of the abnormal cells to be determined rapidly: **(a)** CLL and MCL both express the B cell antigens CD19 and CD20 in addition to CD5, which is more usually found, on T cells; **(b)** CLL has a distinct immunological profile compared with other lymphomas (including MCL). The diagnosis can usually be made using these features and the blood film appearances.
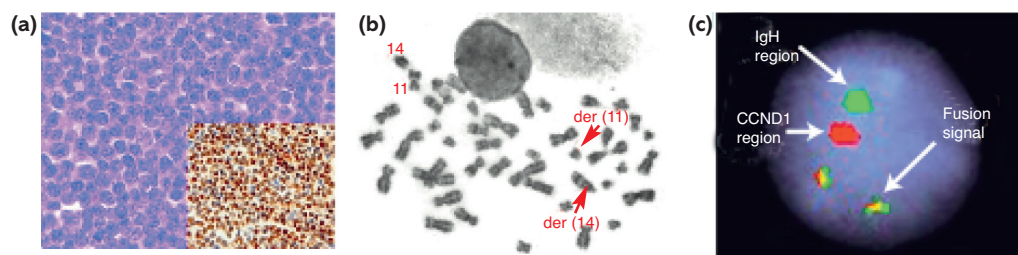
Fig 6. Chronic lymphocytic leukaemia (CLL) and mantle cell lymphoma (MCL). Further investigations on either bone marrow or lymph node tissue useful to confirm the diagnosis of MCL: (a) lymph node biopsy showing strong nuclear staining for Cyclin D1, which is expressed in the abnormal B cells because of translocation of the CCND1 gene from chromosome 11 to the immunoglobulin heavy chain (IGH) gene on chromosome 14; **(b)** metaphase cytogenetics from a patient with MCL showing the t(11;14) translocation; **(c)** detection of the t(11;14) translocation by fluorescence *in situ* hybridisation. This technique may be applied to fixed tissue sections whereas metaphase cytogenetics requires viable tumour cells.

serves populations larger than 500,000. These teams must include a haematopathologist so that the diagnostic information can be integrated with the clinical picture.

The organisational and financial implications of these recommendations have been considerable and inevitably some networks are more advanced than others in their implementation.

The future

The methods available for lymphoma diagnosis are currently evolving rapidly. Molecular techniques including GEP may soon become fully integrated into clinical practice, although further validation is required in prospective studies. The technique is necessarily limited by the need for high quality fresh tissue samples and the cost and complexity of the method. Information gained from GEP studies will guide the development of new antibody panels that identify individual entities and prognostic subgroups. The development of techniques for examining small numbers of cells such as multiparameter flow cytometry and FISH should enable smaller tissue samples or fine-needle aspirates to yield a reliable diagnosis. These improvements in diagnostic precision and risk group delineation should assist clinical decision making and enable targeted therapies to be delivered more rationally.

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