1 General Introduction

The biopsy

The decision on whether or not to biopsy a lymph node or other tissue for a suspected haematological disease is taken by the clinician. In a good haematological oncology unit the pathologist will be involved in this decision, if only to provide technical advice. The correct biopsy optimally handled is very much in everyone's best interests and is often crucial in reaching an accurate diagnosis. The old military adage of the six Ps (proper preparation prevents p*** poor performance) was never more apt. Clinical details of who, when and what are better placed in clinical books but a few practical comments may be appreciated.

Sutton's law

If a patient has significant lymphadenopathy associated with other signs and symptoms, do not mess about with skin, bone marrow smears or cytological preparations in the hope that it will save a biopsy. If patients are sick they need a diagnosis and the money is in the lymph node.

Can we manage with a needle biopsy – skinny or cutting?

In the first edition of this work we said no. These techniques are marvellous in their place but no substitute for a lymph node biopsy when it can reasonably be obtained. It has become increasingly obvious as costs and clinical workloads soar worldwide that we are going to have to cope with needle biopsies more and more whether we like it or not. This means that we will need to rely increasingly on ancillary techniques to make diagnoses. We should, however, never forget that these are suboptimal specimens and be aware that we may have missed the true lesion.

Must we take out the whole node? Can't you make do with a slice?

Yes and no (well perhaps). Lymphoid diseases are often focal and surrounded by reactive changes and so easy to miss on smaller samples. Slices get crushed with resultant misleading artefacts.

Go for the biggest node accessible. Cervical nodes are best and inguinal worst (due to the inevitable reactive changes). Take out the whole node and send to an alerted pathologist or experienced laboratory.

It is much better to send a fresh node so that it can be dealt with promptly and optimally. This also allows material to be used for techniques that can be suboptimal on fixed material such as some molecular investigations. It also enables material to be taken for biobanking, which is becoming increasingly important with the current renewed interest in translational medical research.

If the biopsy cannot be sent or processed that day, get the surgeon or lab technician to slice it like a boiled egg into fixative (plenty of). There is no better way that we know of ruining a lymph node than to ram it whole into a small pot. The capsule is immensely impervious to fixative, leaving the innards to rot rapidly.

Take representative blocks, one per centimetre should easily suffice, send a piece to microbiology if infection is queried and freeze a piece in liquid nitrogen (it can be stored at −70°C later). The rest of the slices can be left in fixative while a diagnosis is being sought. Most hospitals keep this material for a month or so, allowing plenty of time to go back for more blocks if necessary.

Which fixative?

Theses have been written on this and largely ignored. Ninety-nine per cent of the world uses formalin and shows

The Diagnosis of Lymphoproliferative Diseases, Second Edition. Kevin C. Gatter, Georges Delsol, Roger A. Warnke, Francesco Pezzella.

little evidence of changing. And if the steps above are taken, it is a good all-round reagent that is hard to better. All the other fixatives suggested to date (e.g. Bouin's, B5, Duboscq–Brasil) are good in experienced hands but may compromise certain antibody stains and most molecular studies.

Immunocytochemistry

If you are doing serious haematopathological diagnoses you will be using a lot of antibodies. So you might as well invest in an immunostaining machine (or two). Once done you will wonder how you coped without it. It is just like having a dishwasher at home. All the machines currently on the market work well. They differ in the degree to whether they are open or closed systems, as well as whether antigen retrieval is available on the machines, or whether it must be performed manually (see below). Closed systems are fine when the finances for immunostaining are no problem because all the reagents can be bought pre-packed from the manufacturer and used straight off the shelf. If every penny (cent or euro) counts (as in the cash-starved NHS in the UK), then completely open systems are best where individual reagents, including the detection reagents, can be bought at good prices or begged and borrowed and used according to local recipes.

To our knowledge all machines are capable of following most techniques but, as with the fixatives above, most of the world has settled on one or other variation of immunoperoxidase staining. This gives excellent reliable results, and full details and technical back-up are available from a range of antibody companies. Recently introduced antigen retrieval techniques have been a revolution, so every laboratory needs a microwave and/or pressure cooker for manual methods as well as for certain staining platforms. Again all the reagents and advice necessary are available from the relevant commercial companies.

Which antibodies?

In the early days of immunocytochemistry considerations of cost and labour led most pathologists to select reagents singly, according to the details of each case. Now that immunostaining is an accepted diagnostic technique and automated machines are available, there is little reason any longer to remain parsimonious. Link this with the current epidemic of medicolegal claims, and comprehensive panels of antibodies start to seem common sense. Ask yourself if you could justify in court missing a mantle cell lymphoma or a reactive node just because you thought a cyclin D1 or a bcl-2 immunostain was not warranted.

Specific details of individual antibodies and how they are used and interpreted are discussed under the individual entities. Here, for reference purposes, are the antibodies that we use routinely. All refer to use on paraffin-embedded fixed material. The clone numbers given are those known to us as working antibodies. New and better reagents appear regularly and may be available from more than one producer, so it is worth looking around. Pre-treatments for optimal staining are constantly being updated. They differ from place to place, and change as new techniques are described and new reagents produced. It is worth each laboratory testing out a number of these for themselves for each new antibody introduced. Table 1.1 gives a differential diagnosis between lymphoma and non-haematopoietic tumours.

Monoclonal antibodies of diagnostic value in paraffin sections in the diagnosis of haematopoietic neoplasms

The antibodies used for different antigens can be organised into panels for specific purposes or divided into those for first and subsequent runs of immunostaining according to personal preference. The importance of the panel approach is to ensure that an important diagnosis is not overlooked. Table 1.2, for example, gives some panels that we use in routinely diagnosing and classifying lymphomas.

Low-grade B-cell lymphoma

CD20, CD79a, CD3, CD10, bcl-2 protein, κ and λ, cyclin D1, CD5, CD23, proliferation marker (Ki67 or equivalent). When a marginal zone lymphoma (MZL) of MALT (mucosa-associated lymphoid tissue) type, add a pancytokeratin.

High-grade B-cell lymphoma

CD20, CD79a, CD3, CD10, cyclin D1, CD5, CD30, proliferation marker (Ki67 or equivalent) and bcl-2 protein.

T-cell lymphomas

CD3, CD4, CD8, CD30, proliferation marker (Ki67 or equivalent) as well as CD20 and antibodies against follicular dendritic cells (FDCs: CD21, CD23, CD35 or CNA.42). Antibodies reacting against CD2, CD5 and CD7 antigens can be useful for demonstrating loss of one or more T-cell-associated antigens.

Hodgkin lymphoma

CD15, CD30, CD20, CD3, MUM1, EMA, LMP-1 and proliferation marker (Ki67 or equivalent).

Important additional markers for haematopathology

ALK-1, EMA, CD21 and other FDC markers, CDw76 (DBA.44) and annexin A1, plasma cell marker (VS38 or CD138-syndecan), CD68 and CD163, and CD1a and langerin.

A range of markers for non-haematopoietic and myeloid proliferations also needs to be available.

Table 1.1 Differential diagnosis between lymphoma and non-haematopoietic tumours (i.e. undifferentiated carcinoma and malignant melanoma)

Antibodies/Clones	Major reactivities	Unexpected reactivities: remarks
CD45	Leukocyte common antigen (LCA) expressed by 90% of B- or T-cell lymphomas	Exceedingly rare
Two clones 2B11 and PD7/26	Some large-cell lymphomas, in particular anaplastic large-cell lymphoma and lymphoblastic lymphoma, are weakly positive or even negative for LCA Erythroblasts and megakaryocytes are negative Reed–Sternberg cells are usually negative	Occasional seminomas or primitive sarcomas positive for CD45 have been reported Exceptional carcinomas may show strong CD45 cytoplasmic staining though this is generally a misinterpretation of macrophages within the tumour
LSP1 Clone: LSP1	Most lymphomas and myeloproliferative disorders	No cross-reactivity has been reported
EMA Clone E29	Epithelial membrane antigen expressed by most carcinomas Various benign or malignant tumours of diverse origin >80% of anaplastic large cell lymphomas	Expressed by : L&H cells (>80%) in lymphocyte predominance Hodgkin disease; 10% of large B- or T-cell lymphomas; erythroblasts
Cytokeratin clones: 1. KL1 2. MNF116 3. CAM 5.2 4. AE1/3	Most carcinomas are positive Numerous non-epithelial neoplasms may be positive: sarcoma, melanomas, etc.	Occasional lymphoma and plasma cell tumours may show a cytoplasmic dot-like staining
HMB45 Clone HMB45	Melanocytic proliferation; malignant melanoma	Reacts with angiomyolipoma of the kidney and clear cell sarcoma
PNL2 Clone PNL2	Melanocytic proliferation; malignant melanoma	Reacts with angiomyolipoma of the kidney and clear cell sarcoma; granulocytes
S100 Polyclonal	Reacts with S100 A and B Most malignant melanomas are positive	Reacts with a wide variety of normal cells and tumours

Pitfalls in immunohistochemistry

Although immunohistochemistry allows the diagnosis of most lymphoid tumours, there are some that remain difficult to identify. Several factors are responsible for diagnostic difficulties and pitfalls in immunohistochemistry, and quality control has often been raised as a critical issue. False-negative staining is more common than false-positive staining (with the notable exception of false-positive staining due to passive absorption of extracellular Ig) and fixation is the most common culprit. The following discussion is restricted to the labelling of paraffin-embedded biopsy specimens

False-negative staining

False-negative staining may be due to denaturation of either antigen or antibody. As far as the denaturation of the antigen is concerned, one must bear in mind that the gold standard in immunohistochemistry is the labelling on frozen sections which preserves all antigens. However, antigens surviving fixative or that can be retrieved using a number of procedures such as microwave oven heating [1] are now well known. Nevertheless, in some tissue specimens, even after using the latter procedure, some antigens cannot be retrieved, mainly because of the length of the fixation or the nature of the fixative or for other unknown reasons. We have noted that, in a given tissue specimen, some antigens are better preserved than others. Fortunately, in most cases, there is an internal control (i.e. normal B lymphocytes for B-cell antigens, plasma cells for Ig or epithelial membrane antigen [EMA] staining) which, if absent, may indicate that we are dealing with a false-negative staining.

Of course, the lack of internal controls may also be related to technical problems involving one or several steps of the staining protocol. Sections stained in the same batch are usually good external controls to rule out such a pitfall. A number of laboratories use positive and negative tissue specimens such as tonsil for B, T and cytokeratin stainings that are subjected to the same immunohistochemical procedure as the samples stained for diagnostic purpose. Sometimes these are mounted on the same slides as the diagnostic sections. This is convenient provided that one is aware of this and does not score the control staining instead of the diagnostic specimen. It is worth noting that a positive staining

Table 1.2 Monoclonal antibodies of diagnostic value

	T-cell markers	
Antibodies/Clones	**Major reactivities**	**Unexpected reactivities: remarks**
CD1a Clone: O10	Reacts with cortical thymocytes, Langerhans cells and interdigitating dendritic cells Of diagnostic value in Langerhans cell histiocytosis Some precursor T-cell lymphomas are also positive	No reported cross-reactivity
CD2 Clone: AB75	Reacts with most T cells Most T-cell lymphomas are positive	<5% of examples of classic Hodgkin lymphoma (CHL) show reactivity in Reed–Sternberg (RS) cells
CD3 (polyclonal) CD3: monoclonal	Intracytoplasmic domain of CD3 ε-chain Reacts with most T-cell lymphomas (>75%) Reacts with cytoplasmic CD3 in about 50% of natural killer (NK) cell lymphomas Frequently negative or weakly positive in anaplastic large cell lymphoma	Exceptional cases of malignant lymphoma express CD3 and CD20 The RS cells in <5% of examples of CHL show a dot-like staining in the Golgi area Some (<10%) cases of acute myeloid leukaemia (AML) are CD3+ Megakaryocytes may show a weak non-specific cytoplasmic staining
CD4 Clone: 1F6 MT310	Reacts with transmembrane glycoprotein found on helper/inducer T cells Good marker for CD4+ T-cell lymphomas	Macrophages are positive for CD4 True histiocytic tumours and Langerhans cell histiocytosis are CD4 + <5% of examples of CHL show reactivity in RS cells
CD5 Clone: 5C7	Reacts with thymocytes and T cells in lymph nodes A small subpopulation of B cells in lymph nodes is CD5+ Most T-cell lymphomas and leukaemias are positive	Antibody of diagnostic value (together with other antibodies for the diagnosis of chronic lymphocytic leukaemia (CLL) (CD5+ in 95% of cases] and mantle cell lymphoma (CD5+ in 90% of cases] Some thymic carcinomas are CD5+
CD7 Clone: CBC37	Expressed by thymocytes and T cells in lymph nodes and spleen Most nodal T-cell lymphomas positive This antigen is frequently absent in cutaneous T cell and some other peripheral T-cell lymphomas	Some myeloid leukaemias are positive
CD8 Clone: C8/144B	Expressed by cytotoxic/suppressor T cells	Useful antibody for labelling lymphomas and leukaemias derived from cytotoxic/suppressor T cells
CD43 Clones: MT1, DF1, Leu-22	Detects sialophorin expressed on the cell membrane of myeloid cells, T cells and some histiocytes and plasma cells Most T-cell lymphomas are positive Most anaplastic large cell lymphomas (CD3–) are positive for CD43 Langerhans cell histiocytosis and true histiocytic neoplasms are positive RS cells in Hodgkin lymphoma are negative	Useful antibody for the diagnosis of mantle cell lymphoma (>50% positive) and marginal zone lymphoma (>25% positive) as well as CLL/small lymphocytic lymphoma (SLL) (80% positive) Precursor lymphoblastic B-cell lymphomas are often positive (60% of cases) Epstein–Barr virus (EBV)-positive large cell lymphomas are often positive
CD45RO Clone: UCHL1	Subpopulation of T cells (memory) and macrophages Most peripheral T-cell lymphomas In autoimmune lymphoproliferative syndrome (ALPS) the CD45RO+ memory T cells are decreased and the CD45RA+ naïve T cells are increased	Some B-cell lymphoma and plasma cells neoplasms are positive Reacts with myelomonocytic cells and granulocytes

Table 1.2 (*continued*)

	T-cell markers	
Antibodies/Clones	**Major reactivities**	**Unexpected reactivities: remarks**
βF1 TCR β chain Clone: 8A3	Reacts with a non-polymorphic determinant of the β chain of the T-cell antigen receptor (TCR) Most T-cell lymphomas expressing αβ receptors are positive for βF1. However, on paraffin sections the staining is strongly dependent on the fixative and length of fixation	RS cells in Hodgkin lymphoma may show a focal paranuclear staining in 10% of cases
TCR γ chain Clone: γ3.20	Recognises γδ T cells in normal and neoplastic conditions	Useful in identifying rare cases of γδ T-cell lymphoma No reported cross-reactions

	B-cell markers	
Antibodies/Clones	**Major reactivities**	**Unexpected reactivities: remarks**
CD20 Clone L26	Phosphoprotein expressed by more than 90% of B-cell lymphomas: Precursor B-lymphoblastic and diffuse large B cell with plasmacytic differentiation may be negative L&H (lymphocytic and histiocytic) cells of LP Hodgkin lymphoma RS cells in classic Hodgkin lymphoma are positive in 30% of cases but staining varies from cell to cell	Occasional T-cell lymphomas are positive Non-specific granular cytoplasmic staining can be found in some macrophages Non-specific nucleolar staining may be observed in a variety of haematopoietic and non-haematopoietic tumours (e.g. in RS cells or in carcinomas) Epithelial cells in thymoma may be CD20+ Rare cases (<5%) of AML are CD20+
CD23 Clone: MHM6, 1B12	Reacts with follicular dendritic cells and a variable proportion of mantle zone lymphocytes Expressed by 90% of B-CLL cases Other neoplasms are usually negative	About 70% of mediastinal large B-cell lymphomas are positive Subset of B-cell lymphomas positive (25% of follicular, 10% of marginal zone, <10% of non-mediastinal large B-cell, 5% of mantle cell)
CD45RA Clone: DBB42	Reacts with B cells in lymph nodes and spleen A subset of T cells (large granular lymphocytes or LGLs) and naïve T cells as well as histiocytes/ macrophages express this antigen Most B-cell lymphomas are positive Naïve T-cells increased in autoimmune lymphoproliferative syndrome	Large granular lymphocytic leukaemias and approximately 20% of other T-cell lymphomas are positive
CD76-like Clone: DBA-44	Reacts with mantle zone lymphocytes and scattered immunoblasts in lymph node pulp Of diagnostic value in hairy cell leukaemia, in particular for detecting minimal or residual bone marrow involvement in patients after treatment diffuse large B-cell lymphomas are frequently positive.	Endothelial cells are weakly positive Erythroblasts may be positive
CD79a Clone JCB 117	Detects mb1 protein associated with surface immunoglobulin (SIg) In contrast to CD20 the staining is mainly cytoplasmic Reacts with virtually all B-cell lymphoma including those with plasma cell differentiation	50% of lymphoblastic T-cell lymphomas are positive Rare cases of other T-cell lymphomas including extranodal NK T-cell lymphoma are positive for CD3 and CD79a Some smooth muscle cells show a cytoplasmic staining
Anti-Ig heavy and light chains	On paraffin sections only cytoplasmic Ig routinely detectable	False-positive staining may be observed on a variety of cells (i.e. RS cells) and tumours because of passive absorption of extracellular Ig

(*continued*)

Table 1.2 (*continued*)

	B-cell markers	
Antibodies/Clones	**Major reactivities**	**Unexpected reactivities: remarks**
PAX5 Clone: NCL-L-PAX5	Pax5 encodes for a B-cell-specific transcription factor that is expressed in pro-B cells and subsequently in all stages of B-cell development until the plasma cell stage in which it is downregulated	Also positive on most RS cells which can be useful in the distinction of CHL from anaplastic large-cell lymphoma (ALCL)
IgD Clone: IgD26	Reacts on paraffin sections with SIgD of small B lymphocytes of mantle zone CLL and mantle cell lymphomas are usually positive	None reported
CD138/Syndecan 1	Reacts with reactive and neoplastic plasma cells Large B-cell lymphomas with plasma cell differentiation are positive	Reacts with a variety of non-haematopoietic neoplasms, in particular squamous cell carcinoma
OCT2 and BOB1 Clones: PT1, sc955	These are two B-cell transcription factors that are generally used together to help distinguish CHL (usually negative) from B-cell lymphomas and nodular lymphocyte-predominant Hodgkin lymphoma (positive)	
VS38 Clone: VS38	Recognises the p63 antigen on ribosomes Reacts strongly with most reactive and neoplastic plasma cells Large B-cell lymphomas with plasma cell differentiation are positive	Reacts with a variety of non-haematopoietic neoplasms, especially endocrine tumours
BCL6	In normal lymphoid tissue BCL-6 is preferentially expressed by germinal centre cells but not by immature B-cell precursors or plasma cells Most diffuse large B-cell lymphomas are BCL-6+ L&H cells in lymphocyte predominance Hodgkin lymphoma are usually positive whereas in only 50% of classic Hodgkin lymphoma RS cells are BCL-6+	BCL-6 is found in CD4+ cells within germinal centres and in scattered T cells in the perifollicular areas Cortical thymocytes and T-cell lymphoblastic lymphomas are often BCL-6+
Annexin A1 Clone: 29	37-kDa calcium and phospholipids-binding protein is a strong inhibitor of glucocorticoid-induced eicosanoid synthesis and phospholipase A_2 Positive on virtually all cases of hairy cell leukaemia but not on any other B-cell lymphomas	Neutrophils, monocytes, macrophages, subset of CD4+ T cells Many carcinomas are positive

	Histiocytic markers	
Antibodies	**Major reactivities**	**Unexpected reactivities: remarks**
CD68 Clone: KP1	Stains macrophages in a wide variety of human tissues and plasmacytoid dendritic cells in lymph nodes Reacts with all true histiocytic tumours Reacts with myeloid precursors and granulocytes Most AMLs show strong cytoplasmic staining	Some B-cell lymphoma leukaemias such as CLL and hairy cell leukaemia may show a weak granular cytoplasmic staining Some ALCLs are positive Reacts with melanomas

Table 1.2 (*continued*)

	Histiocytic markers	
Antibodies	**Major reactivities**	**Unexpected reactivities: remarks**
CD68 Clone: PGM1	Stains macrophages in a wide variety of human tissues and plasmacytoid dendritic cells in lymph nodes Reacts with all true histiocytic tumours but only M4 and M5 AMLs are positive	in contrast to KP1 this antibody does not cross-react with myeloid cells acute myeloid leukaemias M1, M2, M3, M6 and M7 types are negative
CD163 Clone: NCL-CD163	CD163 antigen is restricted in its expression to the monocytic/macrophage lineage. It is present on all circulating monocytes and most tissue macrophages	Exceptions are macrophages found in the mantle zone and germinal centres of lymphoid follicles. It does not stain interdigitating reticulum cells or Langerhans cells
Lysozyme	Stains macrophages in a wide variety of human tissues Reacts with all true histiocytic tumours Acute and chronic myeloid leukaemias	Frequent background staining
S100 protein	Stains Langerhans cells and interdigitating reticulum cells Virtually all cases of Langerhans cell histiocytosis are positive	Many non-haematopoietic neoplasms express this antigen, especially melanoma
CD207 Langerin Clone: 12D6	Langerin (CD207) is a type II membrane-associated C-type lectin known to be expressed exclusively by Langerhans cells. Langerin recognises mannose residues via its single carbohydrate recognition domain (CRD)	Occasional focal staining in non-Langerhans cell histiocytic sarcomas

	Activation and proliferation markers	
Antibodies	**Major reactivities**	**Unexpected reactivities: remarks**
CD30 Clone: Ber-H2 or JCM182	Glycoprotein of the tumour necrosis factor/nerve growth factor (TNF/NGF) receptor family Activated B or T cells RS cells in classic Hodgkin lymphoma show membranous and paranuclear cytoplasmic staining. Positivity restricted to the paranuclear area is seen in some cases which may be related to fixation Anaplastic large-cell lymphomas Some large T- or B-cell lymphomas	Embryonal carcinoma With some fixatives plasma cells (BerH2) or granulocytes (JCM182) may show cytoplasmic staining Rare undifferentiated squamous carcinomas are positive Mastocytosis is positive About 70% of mediastinal large B-cell lymphomas are positive
Ki-67/MIB1	Reacts with a nuclear antigen expressed in G1, S, G2 and M phases of the cell cycles Useful antibody to assess the growth fraction rate in lymphoproliferative disorder; In some overfixed specimens the staining may be restricted to nucleoli or mitotic figures	None reported consistently

(*continued*)

Table 1.2 (*continued*)

	Myeloid, erythroblast and megakaryocyte markers	
Antibodies	**Major reactivities**	**Unexpected reactivities: remarks**
CD15 Various clones	Detects the X hapten, a late differentiation marker of myeloid cells, also expressed by some monocytes and epithelioid cells Membranous and paranuclear cytoplasmic staining. Positivity restricted to the paranuclear area is seen in some cases which may be related to fixation Useful in the diagnosis of Hodgkin lymphoma: 80% of cases are positive Some T-cell lymphomas and occasional B-cell lymphomas are positive About 5% of anaplastic large-cell lymphoma are positive but the staining is weaker than that of RS cells	This antigen is expressed by many normal and malignant epithelial cells. Cytomegalovirus (CMV)-infected cells label for CD15
CD31 Clone: JC70	Endothelial cells, platelets and megakaryocytes Some reactive and neoplastic plasma cells and histiocytes are labelled	Vascular tumours
Myeloperoxidase	Granulocytes and their precursors Useful markers for the diagnosis of extramedullary myeloblastic proliferations AMLs are positive	Systemic and skin mast cell proliferations are positive Some macrophages, e.g. in Kikuchi disease, are also positive
Anti-von Willebrand factor Clone: F8/86	Reacts with von Willebrand factor in megakaryocytes (cytoplasmic staining) Megakaryoblastic leukaemia (M7)	Endothelial cells
CD61 GpIIIa	Megakaryocytes and platelets Megakaryoblastic leukaemia	Reacts with endothelial cells and osteoclasts
Elastase Clone: NP57	Reacts with neutral protease of granulocytes and their precursors Most AMLs are labelled	A few monocytes are weakly labelled

	Follicular dendritic cells (FDCs)	
Antibodies/Clones	**Major reactivities**	**Unexpected reactivities: remarks**
CD21 Clones: IF8, 2G9, DR53	Reacts with C3d complement receptor Stains the FDC network in normal and neoplastic lymphoid tissue	Also positive on a small number of B-cell lymphomas of all types and some cases of T-cell lymphoblastic Positive in many littoral cell angiomas
CD35 Clones: Ber-Mac-DRC, C3br, TO5	Reacts on paraffin sections with the C3b receptor of complement (CR1) Stains the FDC network but the reactivity is strongly dependent on the type and length of fixation	Reacts with epithelial cells of renal glomeruli Positive on epithelial cells in inflammatory conditions and many carcinomas
CD23 Clones: MHM6, 1B12	Reacts with follicular dendritic cells and a variable proportion of mantle zone lymphocytes	Expressed by B-CLL and some follicular, marginal zone and infrequent mantle cell lymphomas About 70% of mediastinal large B-cell lymphomas are positive Other neoplasms are usually negative

Table 1.2 (*continued*)

	Follicular dendritic cells (FDCs)	
Antibodies/Clones	**Major reactivities**	**Unexpected reactivities: remarks**
CNA-42 (NC) Clone: CNA.42	Reacts with FDCs and scattered mononuclear cells in the lymph node pulp Some cortical thymocytes are positive useful for demonstrating FDC meshworks in follicular lymphoma and some T-cell lymphomas FDC sarcomas are positive as well as some inflammatory pseudotumours of FDC origin	Mast cells, endothelial cells and a subpopulation of plasma cells are labelled Anaplastic large-cell lymphomas are positive (60%) 40% of T-cell lymphomas are positive weak-to-moderate cytoplasmic staining is found in 20% of cases of Hodgkin lymphoma rare cases of large B-cell lymphoma (5%) are positive

	Miscellaneous	
Antibodies	**Major reactivities**	**Unexpected reactivities: remarks**
BCL-2 Clones: 124, 3.1	Protein able to suppress cell death by apoptosis B and T small lymphocytes are positive but germinal centre cells in reactive lymph nodes are negative Very helpful in the diagnosis of follicular lymphoma (85% +) versus follicular hyperplasia; Most B- or T-cell lymphoma are positive RS cells in Hodgkin lymphoma are frequently positive (60% of cases)	Most AMLs and chronic myeloid leukaemia (CML) in blast crisis are positive Blasts in refractory anaemia with excess of blasts are positive Many normal and malignant epithelial cells are positive
Cyclin D1	Reacts with cyclin D1 antigen Nuclear staining very helpful in diagnosing mantle cell lymphoma	Hairy cell leukaemias and many cases of myeloma are positive Cytoplasmic staining is not considered to be specific
ALK Clone: ALK1	Highly specific for the NPM/ALK protein associated with the t(2;5) and full-length ALK protein Reacts with 85% of anaplastic large cells lymphoma of T or null phenotype Cytoplasmic, nuclear and nucleolar staining is highly indicative of the t(2;5) (90% of cases) 20% cases show a staining restricted to the cytoplasm. These cases are associated with ALK variant translocations (TPM3-ALK, TFG-ALK, ATIC-ALK, etc.)	Rare cases of large B-cell lymphoma (CD30–, EMA+, IgA+) show ALK-positive staining restricted to the cytoplasm of malignant cells Some inflammatory myofibroblastic tumours associated with TPM3-ALK, TPM4-ALK, CLTC-ALK or RanBP2-ALK protein show ALK-positive staining restricted to the cytoplasm of neoplastic cells
TIA1	Granule-associated cytotoxic protein of cytotoxic T cells, NK cells and granulocytes Granular cytoplasmic staining is seen in some peripheral T-cell lymphomas, in particular in anaplastic large-cell lymphoma	Occasional cases of classic Hodgkin lymphoma show positive RS cells As a result of granulocytes labelling in bone marrows, another marker for cytotoxic granules such as granzyme B may be preferred in this setting
MUM1 Clone: MUM1p	Labels MUM1 protein in a subset of light zone germinal centre (GC) B cells (probably centrocytes and their progeny), plasma cells, activated T cells and a wide spectrum of haematolymphoid neoplasms derived from these cells; MUM1 protein is a useful tool for identifying RS cells in classic Hodgkin lymphoma as virtually all are positive	Of non-haematolymphoid neoplasms, only a proportion of melanomas are labelled

(*continued*)

Table 1.2 (*continued*)

	Miscellaneous	
Antibodies	**Major reactivities**	**Unexpected reactivities: remarks**
Perforin	Granule-associated cytotoxic protein of cytotoxic T cells Granular cytoplasmic staining is seen in some peripheral T-cell lymphomas in particular in anaplastic large-cell lymphoma	Useful antibody in the differential diagnosis between neoplastic cell-rich Hodgkin lymphoma (perforin negative) and anaplastic large cell lymphoma (frequently positive)
Granzyme B	Granule-associated cytotoxic protein of cytotoxic T cells Granular cytoplasmic staining is seen in some peripheral T-cell lymphomas	
TdT Rabbit anti-TdT	Reacts with terminal deoxynucleotidyl transferase Most cortical thymocytes are positive (nuclear staining) Small population (<1%) of bone marrow lymphoid cells Most precursor B- and T-lymphoblastic leukaemia/lymphoma is TdT+	Exceptionally found in a few cells in reactive lymph nodes, tonsils and spleens Cytoplasmic staining is considered to be non-specific Some cases of AML are positive
CD10	Positive on most B lymphoblastic lymphoma/leukaemias and some T lymphoblastics Most germinal centre cells are positive as are about 80% of follicular lymphomas Some large B-cell lymphomas are positive	Stromal cells in a variety of tissues are positive, making a useful internal control Angioimmunoblastic T-cell lymphomas are often labelled for CD10 but newer markers such as BCL6, CXCL13 and PD1 more often label these follicular helper T cells
CD34 Clone: QBEN10	Glycoprotein expressed by lymphohaematopoietic stem and progenitor cells Reacts with precursor B- or T-lymphoblastic lymphoma/leukaemia AMLs are also labelled	Endothelial cells Vascular tumours Some sarcomas
CD56	reacts with neural cellular adhesion molecule (NCAM) NK neoplasms in particular T/NK cell nasal lymphoma	CD56 is expressed by 20% of AML and in myeloblasts of high-grade myelodysplastic syndromes
CD57 Clones: IOT10 anti-Leu7	Carbohydrate antigen expressed on mononuclear cells with NK activity Some T-lymphoblastic lymphomas and NK tumours are positive Occasional large B-cell lymphomas are positive Lymphocytes forming rosettes around lymphocyte-predominant (LP) cells in LP Hodgkin lymphoma are often CD57+	Reacts with a variety on non-haematopoietic neoplasms such as prostatic carcinoma and neuroendocrine tumours
Anti-HLA-DR Clone: DK22	Expressed by most B-cell lymphomas Occasional T-cell lymphomas developed from activated T cells are positive Langerhans cell histiocytosis is positive Histiocytic tumours are usually positive	Various non-lymphoid tumours are positive for HLA-DR
c-kit (CD117)	Mast cells and mastocytosis are strongly positive Many cases of AML and blast cells in myelodysplasia are positive	Various lymphoid and non-lymphoid tumours (e.g. gastrointestinal stromal tumours or GISTs) are positive for c-kit

Table 1.2 (*continued*)

	Miscellaneous	
Antibodies	**Major reactivities**	**Unexpected reactivities: remarks**
Vimentin Clone V9	Occasional B- or T-cell lymphomas are positive RS cells in Hodgkin lymphoma are usually positive	Expressed by a wide variety of benign and malignant cells
Fascin Clone: K-2	Reacts with actin-bundling protein expressed by dendritic cells Stains dendritic cells (interdigitating reticulum cells) in lymph nodes RS cells in classic Hodgkin's disease are usually positive (L&H cells are negative) Langerhans cell histiocytosis is positive	FDCs are weakly to moderately positive Endothelial cells are strongly positive Occasional B- or T-cell neoplasms are positive

	Infectious agents	
Antibodies	**Major reactivities**	**Unexpected reactivities: remarks**
p24 Clone: Kal-1	HIV type 1 capsid protein Reacts with the p24 protein in FDCs infected by HIV	Background staining may be responsible for false positive staining
CMV Clones: 2A2, E13	Reacts with early antigen (EA) detected during the CMV infection	
LMP1 Clone: CS1-4	Reacts with EBV latent membrane protein in latently infected cells Immunoblasts in infectious mononucleosis are LMP1+ Many cases of classic Hodgkin lymphoma are positive Most large B-cell lymphomas in HIV-positive patients and post-transplantation B-cell lymphomas are positive Scattered non-neoplastic B-cell immunoblasts are found in some peripheral T-cell lymphomas T/NK cell lymphoma latently infected by EBV (i.e. nasal-type lymphomas) are usually negative (but positive for EBV RNA or EBER)	Reactive plasma cells may show a non-specific cytoplasmic staining In some bone marrow biopsies megakaryocytes may show a strong cytoplasmic staining Extensive non-specific labelling may be observed in B-5-fixed sections
EBNA2 Clone: PE2	EBV nuclear antigen expressed in latently infected cells EBV-positive, large B-cell lymphomas express this antigen EBV-positive RS cells are negative for EBNA2	On paraffin section the staining is usually weak
HHV8 Clone: LN53	This antibody reacts with the latent nuclear antigen (LNA-1) of human herpesvirus 8 (HHV8) Kaposi sarcoma and lymphoid cells in multicentric Castleman disease are positive	Positive in primary effusion lymphomas as well as the large B-cell lymphomas that arise in HHV8+ multicentric Castleman disease

of the control samples and a negative staining of the tested sample do not exclude the possibility of false staining because the various steps in the preparation of the tissue sample (including length of fixation and type of fixative) may be different. In addition, control tissue on the same slide may be falsely negative on some staining platforms if control tissue is not placed close enough to the target tissue because reagents may not cover the entire slide. Once again, the presence of an internal positive control is the most reliable criterion to decide whether neoplastic cells in a given biopsy specimen are negative or not. Finally, false-negative staining may be due to antibodies that are not suitable for immunohistochemistry even though they are perfectly good reagents for other techniques such as western blotting.

False-positive staining

If one excludes background staining, non-specific labelling may be related to contaminating antibodies in the primary serum (i.e. anti-IgG reactivity in an anti-IgM antiserum). Such non-specific staining may be decreased by increasing the dilution of the primary antibody. The most common false-positive staining in lymphoid pathology is due to passive absorption of extracellular proteins (i.e. Ig and other serum proteins) by neoplastic cells [2]. In some malignant lymphoid proliferations this phenomenon may be responsible for an erroneous diagnosis of a reactive disorder on the basis of a false polytypic staining (i.e. lymphoma cells positive for Igκ and Igλ) because neoplastic cells have passively absorbed IgGκ and IgGλ, which are in large amounts in the serum and thus in the extracellular milieu. For unknown reasons, large cells in diffuse large B-cell lymphoma and Reed–Sternberg cells or even histiocytes have a great tendency to absorb passively extracellular proteins. Some signs may allow the recognition of false-positive staining due to passive absorption:

- The staining is diffuse rather than granular and the nucleus is often stained.
- Positive cells are scattered among a majority of negative cells.
- The artefactual staining is proportional to the concentration of proteins in the serum (IgG > IgM).
- Double staining for κ and λ reveals mixed staining
- J-chain staining may be helpful for differentiating positive Ig-secreting cells (J chain positive) from cells having passively absorbed extracellular Ig (J chain negative).

Unfortunately there is not at present a good commercially available anti-J chain antibody for use on routine specimens.

Unexpected reactivity of monoclonal antibodies

Some antibodies, which are very useful in routine immunohistochemistry, may show a very confusing, unexpected reactivity with some normal or neoplastic cells. One of the best examples of this phenomenon is represented by the reactivity of anti-EMA antibodies.

Anti-EMA antibodies

Anti-EMA usually refers to the commercially available anti-EMA/E29 antibody, although there are other antibodies reacting with epithelial membrane antigen. Positive staining of a subset of plasma cells was recognised early in the history of EMA and now constitutes an acceptable internal control in haematolymphoid tissues [3]. The staining is mainly membrane associated. The most consistent reactivity of EMA within B-cell neoplasms is found in multiple myeloma and plasmacytoma [4]. In hyperplastic bone marrow showing increased numbers of immature cells, some erythroblasts may react with anti-EMA antibody. However, the membrane staining is usually weak, and sometimes the staining is restricted to the Golgi area (dot-like staining). Caution is therefore required when using anti-EMA antibodies for detecting marrow involvement in epithelial malignancies or in patients with anaplastic large-cell lymphoma (see below). Blastic cells in erythroleukaemia and megakaryoblastic leukaemia may also express EMA.

The most frequent non-epithelial expression of EMA is recorded in the LP cells of nodular lymphocyte-predominant Hodgkin lymphoma and the cumulative experience of the reactivity of LP cells with anti-EMA approaches 80% of cases [5–7]. In cases with apparently negative atypical cells, comparison with the staining intensity of plasma cells as the internal control is critical. Many cases of transformation of nodular lymphocyte-predominance Hodgkin lymphoma to large B-cell lymphoma have been shown to conserve EMA expression [6]. The situation is quite different in classic Hodgkin lymphoma of nodular sclerosis and mixed cellularity subtypes. The reactivity of anti-EMA with Hodgkin and Reed–Sternberg cells and variants is exceedingly rare at less than 5% [4]. The percentage of cases with EMA+ Reed–Sternberg cells is even lower if only the larger reported series are taken into account which suggests that the finding of EMA+ Reed–Sternberg cells and variants should lead to a reassessment of a diagnosis of classic Hodgkin lymphoma. The series of Filippa et al. [8] of 128 cases of nodular sclerosis and mixed cellularity subtypes without EMA expression is notable, and the authors attached clinical significance to the non-expression of EMA in classic Hodgkin lymphoma. Nevertheless, there are probably rare cases where the diagnosis of classic Hodgkin lymphoma is sustainable by morphology and other antibodies (CD15+, CD30+, MUM1+, null cell phenotype) despite the expression of EMA.

Within the lymph node-based, low- and high-grade, B-cell lymphomas, reactivity of EMA is of the order of 5% in major series. EMA is mainly expressed by T-cell-rich B-cell lymphomas and by diffuse large B-cell lymphomas. A distinct subtype of large B-cell lymphoma expressing EMA strongly

together with the tyrosine kinase receptor ALK has been recognised [9].

The presence of EMA on T-cell lymphomas is of the order of 10–20% in larger series [3]. Most cases of enteropathy-associated T-cell lymphoma and anaplastic large-cell lymphoma express EMA [10]. EMA expression in CD30+ primary cutaneous lymphoproliferative disorders is still the subject of controversy. In our experience EMA is expressed by most CD30+ tumours and is not of diagnostic value for differentiating secondary from primary CD30+ cutaneous lymphomas.

Anti-CD30/Ber-H2 antibody

The reactivity of this antibody was first reported in reactive B and T immunoblasts, Reed–Sternberg cells, and is now the defining marker of anaplastic large cell lymphoma [11]. A subpopulation of plasma cells is reactive for CD30/Ber-H2 antibody in formalin-fixed tissue, although this is not seen when heat-associated antigen retrieval methods are used [11]. However, the range of neoplastic cells expressing the CD30 antigen is wider than initially thought [12]. Mastocytosis is commonly positive for CD30 and the authors have observed that occasional cases of true histiocytic tumours and erythroleukaemia are also positive for this antigen.

Some non-haematopoietic cells are positive for CD30/Ber-H2. In addition to the well-known reactivity of embryonal carcinoma, various carcinomas and occasional melanomas may show a reactivity for CD30/Ber-H2(12).

A new clone, JCM182, specifically produced to improve the recognition of CD30 on paraffin sections has recently become available. It too has a few unexpected reactions such as identifying granulocytes especially in bone marrow specimens.

Miscellaneous unexpected reactivities

Some of these reactivities are commonly seen in routine practice and are not really a source of error:

CD20/L26

The nucleolar staining of large number of cells of many different tissue types with CD20/L26 is commonly observed and does not indicate a B-cell origin of these cells. Only membrane-associated staining should be taken into consideration. Rare cases of CD20+ T-cell lymphoma have been reported [13]. CD20 antigen has been reported in occasional cases (<5% of cases) of acute myeloid leukaemia and in epithelial cells of thymoma [14]. Some follicular dendritic cells are also reactive for CD20.

CD79a/JCB117

Unexpectedly, approximately 40% of lymphoblastic T-cell lymphomas are positive for CD79a [15]. However, despite the expression of CD79a, lymphoblastic lymphomas that are positive for CD3 and CD79a are of T-cell lineage [16]. Occasional cases of extranodal NK/T-cell and peripheral T-cell lymphoma may express CD79a antigen [17,18]. The reported positivity of acute promyelocytic leukaemia occurs only with the clone HM57 and not with the more commonly used reagent JCB 117 [19].

CD3

In various lesions, macrophages may show a spurious globular cytoplasmic staining. The authors have observed exceptional cases of B-cell lymphoma with clonal Ig gene rearrangement positive for CD3. Acute myeloid leukaemias express CD3 (as well as other T-cell-associated antigens) in less than 10% of cases.

CD5

This pan-T-cell antigen is expressed by a wide variety of B-cell lymphomas including small lymphocytic lymphoma/leukaemia, mantle cell lymphoma and occasional cases of diffuse large B-cell lymphomas. CD5 is also found in thymic carcinomas and other non-lymphoid neoplasms [20].

CD10

CD10 antibodies recognize a 100-kDa cell surface glycoprotein that is present in a variety of cell types. Originally this antigen was referred to as 'common acute lymphoblastic leukaemia antigen' or CALLA, because it was found to be expressed on the cell surface of most cases of acute lymphoblastic leukaemia (ALL) and thought to be specific for this lymphoproliferative disorder. CD10 antigen can be demonstrated not only on precursor B-cell or T-cell lymphoblastic lymphomas/leukaemias but also on follicular lymphomas, most Burkitt lymphomas and diffuse large B-cell lymphomas (30% of cases). CD10 is also expressed in a wide variety of non-neoplastic human cells, including haematopoietic (germinal centre cells and mature granulocytes), epithelial (renal proximal tubules, bile canaliculi) and stromal cells (fibroblasts).

CD23

The CD23 molecule is a low-affinity IgE receptor found on B cells. It is a membrane glycoprotein of 45 kDa and is reported to be found on a subpopulation of peripheral blood cells, B lymphocytes and Epstein–Barr virus (EBV)-transformed B-lymphoblastoid cell lines. Expression of CD23 antigen has been reported on monocytes and dendritic cells and is generally positive on most cases of B-CLL (B-cell chronic lymphocytic leukaemia). Although originally considered to be negative it has become clear that approximately 20–30% of follicular lymphomas are positive and increasingly positive cases of other subtypes of B-cell lymphoma are being recognised [21].

Anti-TdT antibody

This antibody is very useful for diagnosing lymphoblastic lymphomas from B- or T-cell origin. However, approximately 10% of myeloblastic leukaemias are also positive for TdT. Furthermore cortical thymocytes are strongly positive so a diagnosis of T-lymphoblastic leukaemia in the thymus needs to be made on more than TdT positivity. In addition, there are increased numbers of TdT+ precursors in regenerating bone marrows, so TdT needs to be used with great caution when monitoring the bone marrow of ALL patients for evidence of minimal relapse.

CD68 antibodies

The two anti-CD68 antibodies, PGM1 and KP1, do not have the same reactivity despite being in the same cluster. Thus, CD68/KP1 reacts with immature myeloid cells whereas CD68/PGM1 has a restricted reactivity with monocytes/macrophages. A significant number of lymphocytic and diffuse large B-cell lymphomas are positive for KP1 [22] as are most melanomas [23,24] whereas PGM1 is unreactive with these tumours.

Loss of leukocyte common antigen

Malignant cells may lose one or more antigens usually found on normal cells of their origin. The best example is provided by malignant lymphomas negative for the leukocyte common antigen CD45.

Most malignant lymphomas expressed the leukocyte common antigen CD45 and are negative for epithelial markers. There are, however, exceptions that are responsible for diagnostic difficulties in immunohistochemistry. According to the largest series in the literature, 10% of diffuse large cell lymphomas, 20% of lymphoblastic T-cell lymphomas and most plasmocytomas/myelomas are negative for CD45 [25–27]. Note that more than a third of anaplastic large-cell lymphomas are negative for CD45, the highest rate of negativity among non-Hodgkin lymphomas. Generally speaking, the larger the cells the weaker the expression of the leukocyte common antigen and in some cases this antigen is detected only on frozen sections. It is worth noting that some of these tumours are usually positive for EMA which may lead to an erroneous diagnosis of undifferentiated carcinoma. Additional markers of B- or T-cell lineage along with CD30 should enable a correct identification of the lymphoid nature of these tumours.

Loss of B- or T-cell-associated antigens

In B-cell lymphoma the loss of both CD20 and CD79a antigens has been reported in rare cases of ALK (anaplastic lymphoma kinase)-positive large B-cell lymphoma secreting IgA [9]. Cases of CD20-positive B-cell lymphomas that became CD20 negative at relapse have been reported after rituximab therapy [17] (Fig 1.1).

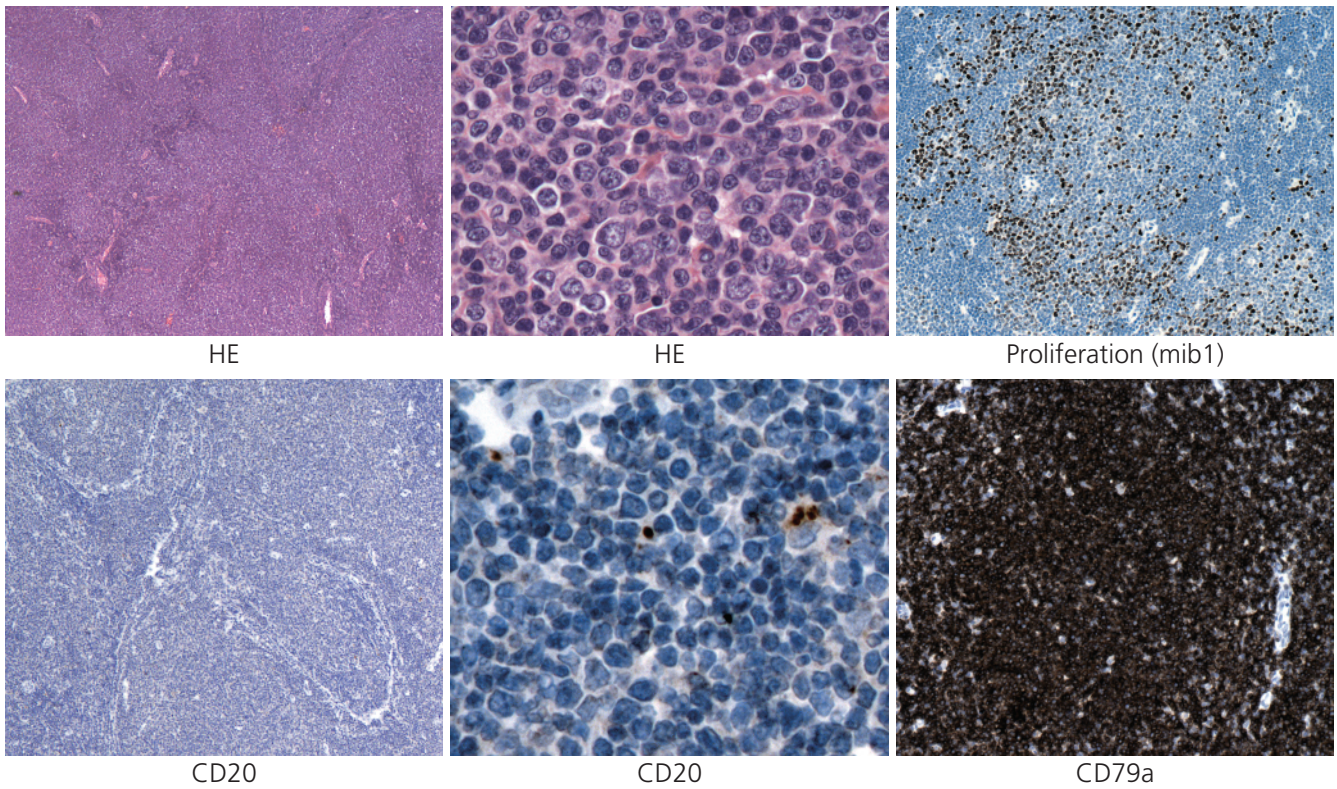

Fig 1.1 Example of a follicular lymphoma that was CD20+ on diagnosis, but at relapse after rituximab therapy is virtually negative apart from a few dots of cytoplasmic staining. The lymphoma remains B cell, being CD79a+, and has the typical cytology and proliferation rate and pattern of a follicular lymphoma.

In T-cell lymphomas the loss of one or more pan-T-cell antigens (CD3, CD5, CD2 or CD7) is seen in most cases (75% of cases), CD7 being the most frequent, particularly in cutaneous T-cell lymphomas [28–30]. Pan-T-cell antigens are commonly missing in anaplastic large-cell lymphomas with CD3 staining being observed in only a third of cases.

Acquisition of antigens

Some malignant cells may express one or several antigens that are normally restricted to another cell lineage. The best example is provided by some lymphomas being positive for cytokeratin or some carcinomas reacting with anti-leukocyte antibodies.

Malignant lymphoma positive for keratin

A number of reports have emphasised the existence of myelomas or large-cell lymphomas positive for cytokeratin [26,31–35]. It is worth noting that, in these cases, the staining for cytokeratin is usually focal and restricted to the paranuclear Golgi area. Of note is that most of these tumours are negative for leukocyte common antigen which may increase the diagnostic difficulty. The diagnosis of lymphoma in these cases relies heavily on the reactivity with anti-T or anti-B antibodies. The authors have also observed rare cases of acute myeloblastic leukaemia positive for cytokeratin.

Other unexpected reactivities

Some other unexpected reactivities have been reported such as B-cell lymphomas reactive for HMB45, the melanoma marker [36], and positivity for S100 protein, actin [37] and vimentin [38]. Unexpectedly, some cases of mediastinal B-cell lymphomas are positive for β human chorionic gonadotrophin (βhCG) [39]. In fact, these aberrant reactivities are rarely encountered in routine practice.

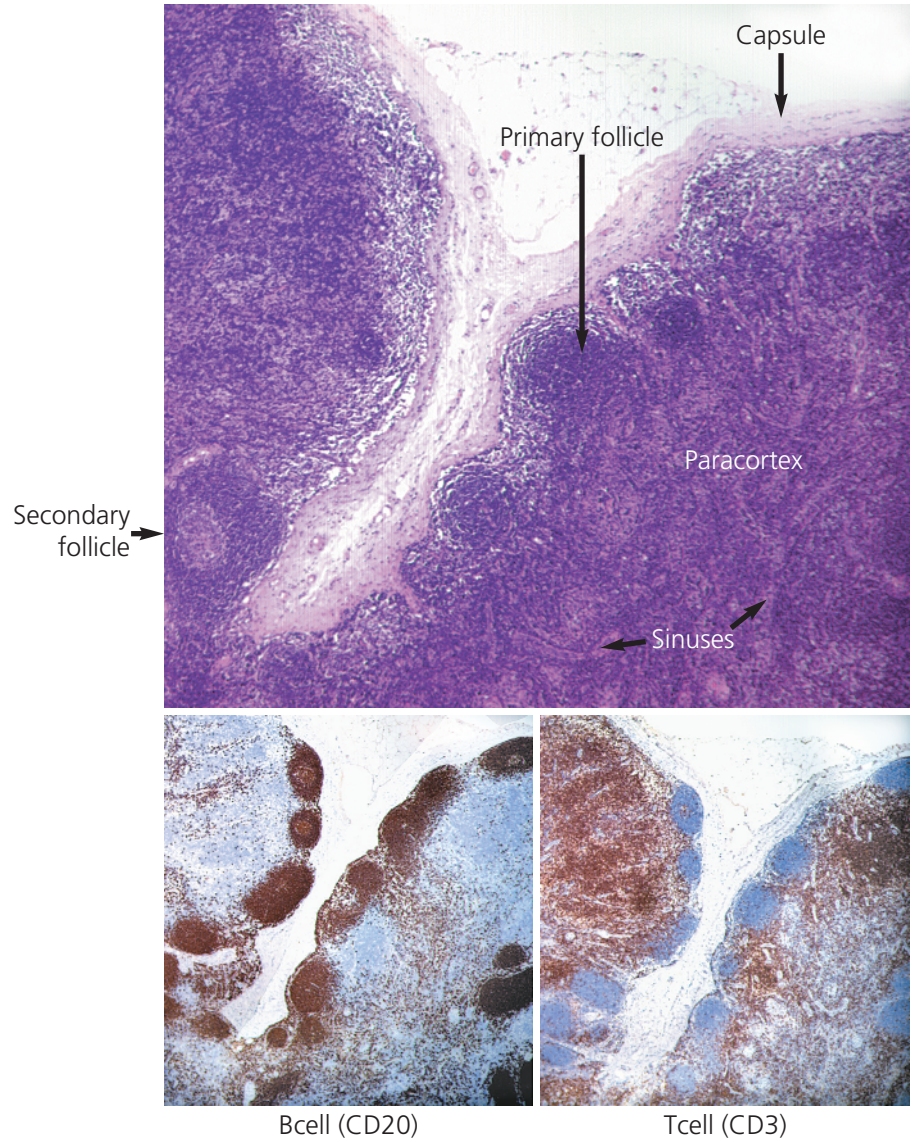

Fig 1.2 Principal structures in a normal lymph node. Normal lymph node structures are indicated. An abundance of primary follicles denotes a lymph node little affected as yet by antigenic stimulation.

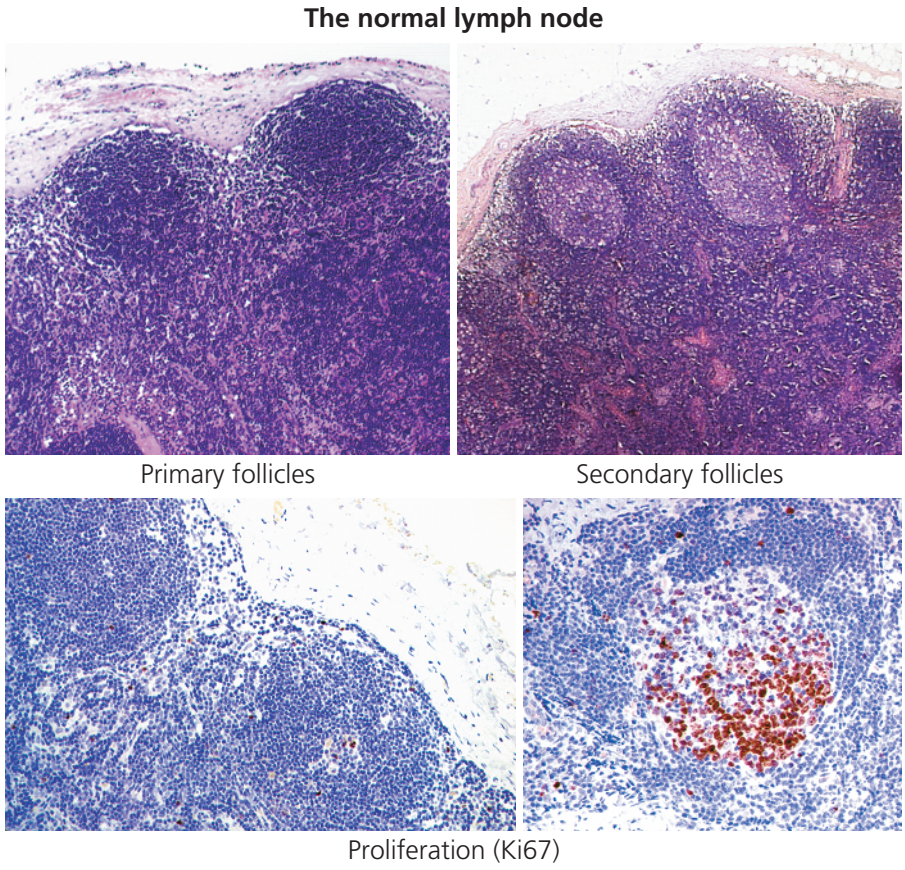

Fig 1.3 When follicles undergo secondary change on antigenic stimulation there is a greatly increased level of proliferation in the developing germinal centre which is predominantly in one area, the so-called 'dark zone' where the centroblasts are most numerous.

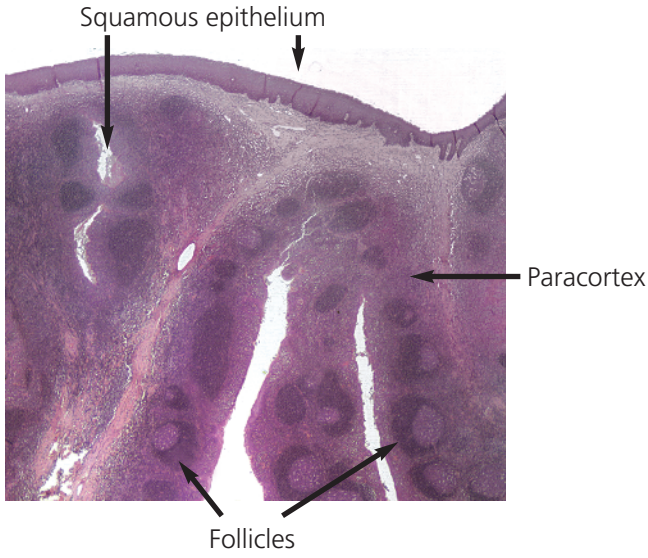

Fig 1.4 Tonsil differs from lymph node principally by being covered by oral-type non-keratinising squamous epithelium. The sinus meshwork is also poorly developed and difficult to identify.

Non-lymphoid tumors positive for the leukocyte common antigen

A more confusing aberrant phenotype is provided by some non-lymphoid tumours positive for the leukocyte common antigen (LCA). Warnke and Rouse [40] reported two cases, one an aggressive pituitary adenoma and the other a seminoma positive for LCA. Such reactivity has also been reported in non-malignant breast lesions [41]. We have observed two cases of breast carcinoma positive for LCA but in these cases the staining was mainly cytoplasmic and not membrane associated as in lymphoid tumours. However, a clear CD45 membrane staining has been reported in undifferentiated and neuroendocrine carcinoma [42]. It should be emphasised that these are exceptionally rare events in diagnostic practice. By far the most common reason for pathologists thinking that a carcinoma is CD45+ is by mistaking a heavy macrophage infiltration for malignant epithelial cells. This can be easily resolved by staining for CD68.

I've only got one H&E – help!

No problem, except you can only do one immunostain so choose carefully. Just immerse the slide in toluene for 12–72 h to remove the coverslip and then place in successive baths of absolute alcohol, distilled water and phosphate-buffered saline. This treatment removes the eosin staining

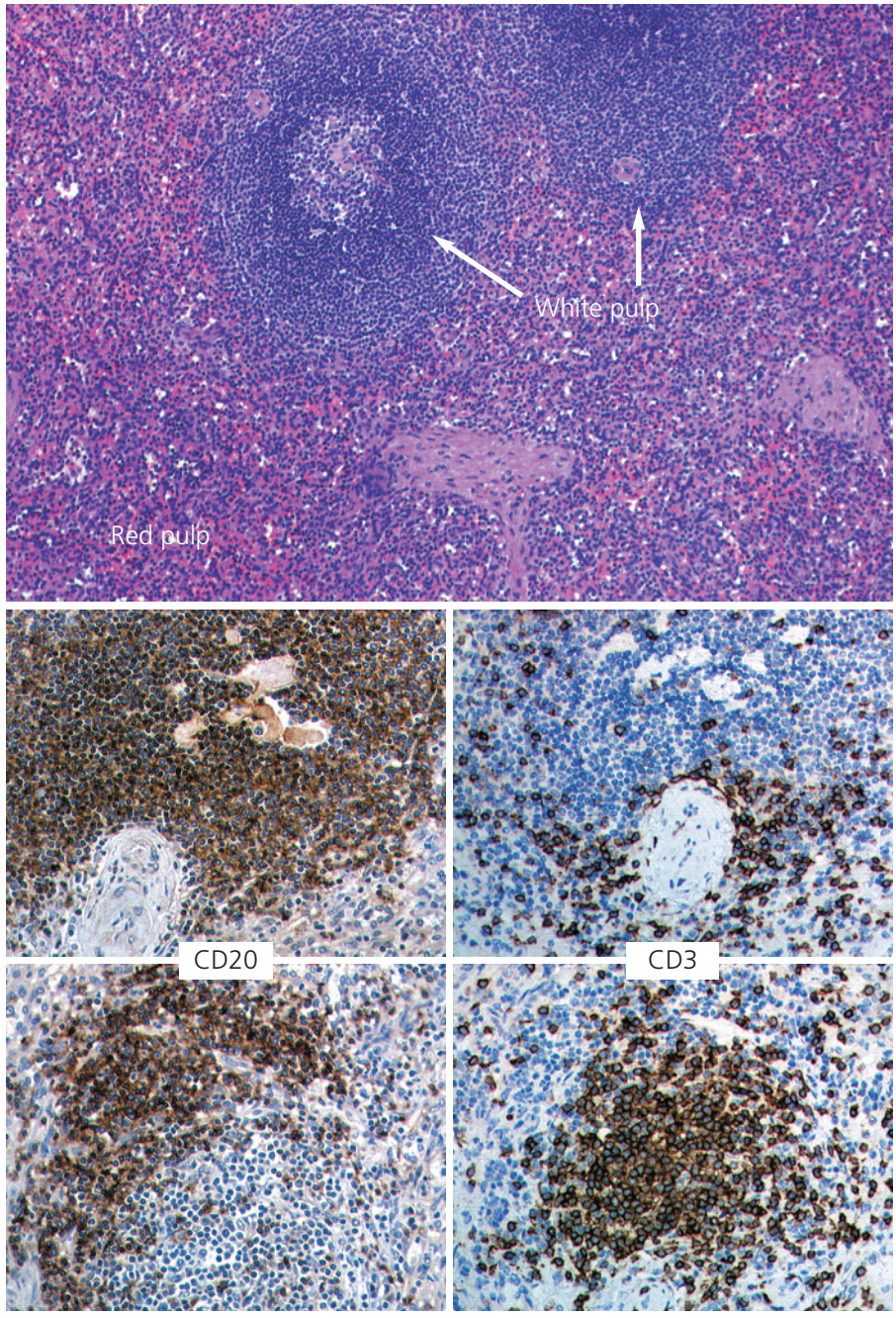

Fig 1.5 In the spleen the lymphoid tissue resides in the white pulp, which similar to lymph node contains well-circumscribed B- and T-cell areas.

of the cytoplasm, but does not affect the nuclear staining. Immunohistochemical staining is then performed as for unstained sections.

Conclusion

This brief description of some pitfalls in immunohistochemistry stresses that the immunophenotypic diagnosis and the cellular origin of a tumour cannot rely on a single immunological marker. Aberrant stainings, such as positive staining for cytokeratin in malignant lymphomas, or lymphomas negative for the LCA (CD45) do not diminish the diagnostic utility of immunohistochemistry, which allows the accurate classification of most if not all haematopoietic neoplasms. However, these unusual stainings emphasise the need to interpret immunohistochemical results with morphology and clinical data.

Molecular techniques

'Molecular studies have revolutionized hematopathologic diagnosis' writes an eminent author of a recent excellent

lymph node textbook [43]. But have they? A positive rearrangement or translocation might be supportive but who feels confident enough to diagnose malignancy when only a band on a polymerase chain reaction (PCR) gel says so? Although these techniques remain expensive they have now become much more reliable in routine service. We continue to use PCR or fluorescent *in situ* hybridisation (FISH) methods as supportive in difficult cases, but do not rely on them for a diagnosis. So, for example, a B- or T-cell clonal rearrangement is helpful in a case with a strong suspicion of B- or T-cell malignancy as is an appropriate FISH translocation for suspected Burkitt lymphoma or mantle cell lymphoma. By themselves they are still insufficiently robust to deliver a diagnosis, although surely similar to telepathology their day will come (though for the moment we continue to wait patiently).

Normal lymphoid structures

The key to virtually all pathological diagnoses is knowing and understanding the normal. It is difficult to define what a normal lymph node is because it is a structure that is changing and adapting daily. Figures 1.2 and 1.3 outline the basic structure of a lymph node, Fig 1.4 the tonsil and Fig 1.5 the spleen, and contrast the difference between an unstimulated node with its primary follicles and one that has been stimulated to produce secondary follicles with germinal centres.

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