Markers and Immunoprofile of Lymphoid Tissue Neoplasms

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Lymphoid tissue is a microenvironment composed of B-, T-, and NK-lymphocytes in different maturation and differentiation stages, plasma cells, macrophages, dendritic cells, reticular cells, and granulocytes. For the diagnosis of lymphoma, all these components must be considered. For initial diagnosis, screening markers are helpful. Further specific markers must be used for the precise diagnosis. Markers listed in different parts of this chapter are essentially used for orientation. The final diagnosis must be done according to the histomorphology, immunophenotype (immunohistochemistry and flow cytometry), and genetic analysis. The 2016 revision of the World Health Organization classification of lymphoid neoplasms was considered in this chapter.

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16.1 Screening Markers for Lymphoma

CD45 (LCA), TdT, B-cell markers, T-cell markers, and Ki-67 [1–3].

CD45 (LCA)		
Expression pattern: membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Lymphoma/ leukemia	Granulocytic sarcoma, histiocytic sarcoma, dendrocytoma, interdigitating dendritic cell sarcoma, giant cell tumor of tendon sheet	Hematopoietic cells including B- and T-lymphocytes, monocytes, macrophages and mast cells, dendritic cells, medullary thymocytes, fibrocytes
Positive control: appendix		

Diagnostic Approach CD45, also known as leukocyte common antigen (LCA), is a family of high molecular mass integral membrane glycoprotein molecules expressed on all hematopoietic cells except mature red cells and their immediate progenitors, megakaryocytes, and platelets.

Diagnostic Pitfalls CD45 is a specific marker for hematopoietic and lymphatic tumors; nonetheless, less than 3% of B-cell lymphoma, about 10% of T-cell lymphoma, and about 30% of precursor B- and T-lymphoblastic lymphomas (ALL) lack the expression of CD45. In suspicious cases, the use of other lymphoid markers is required. Membranous CD45 expression is reported in very rare cases of undifferentiated, neuroendocrine, and small cell carcinomas. Necrotic carcinomas can also imitate a membranous LCA positivity, which also holds true for other markers, as in general, necrosis may display a false positivity.

TdT (Terminal deoxynucleotidyl transferase)		
Expression pattern: nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
B- and T-ALL	AML, CML, Merkel cell carcinoma	B- and T-cell precursors, cortical thymocytes
Positive control: ALL		

Diagnostic Approach Terminal deoxynucleotidyl transferase (TdT) is a DNA nuclear polymerase, catalyzing the template-independent polymerization of deoxynucleotidyl triphosphates to double-stranded gene segment DNA. TdT is mainly expressed in precursors of B- and T-lymphocytes. Therefore, antibodies to TdT are specific markers for precursor cell lymphomas of T- and B-cell origin, namely, acute lymphoblastic leukemia.

Diagnostic Pitfalls It is important to consider that TdT may be positive in some types of acute myeloid leukemia especially minimally differentiated AML (M0) and blast crisis of chronic myeloid leukemia (CML). Furthermore, the TdT expression is characteristic for the immature T-lymphocytes associated with the thymoma types A, B, and AB but not thymic carcinoma.

TdT is also positive in a large percentage of Merkel cell carcinoma, which may be also positive for PAX-5 [4, 5].

CD5 and CD10 are further markers for the diagnosis and classification of lymphomas. Both do not have lineage specificity and may be expressed in both B- and T-cell lymphomas in addition to other nonlymphoid neoplasms.

CD10 (CALLA)		
Expression pattern: membranous/cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Burkitt lymphoma, acute lymphoblastic lymphoma/leukemia, angioimmunoblastic T-cell lymphoma, endometrial stromal tumors, renal cell carcinoma	Follicular lymphoma, plasma cell neoplasms, hepatocellular carcinoma, transitional cell carcinoma, colorectal adenocarcinoma, prostatic carcinoma, melanoma, placental site trophoblastic tumor, choriocarcinoma, myofibroblastoma, mesothelioma, rhabdomyosarcoma, leiomyosarcoma, Ewing's sarcoma, solitary fibrous tumor, atypical fibroxanthoma	Pre-B and pre-T cells, cells of germinal centers, granulocytes, adrenal cortex, endometrial stroma cells, hepatocytes and bile duct canaliculi, cells of proximal renal tubules and glomerular epithelial cells, endothelial cells, myoepithelial cells, fibroblasts, brain tissue, choroid plexus, fetal intestinal epithelium, mesonephric remnants
Positive control: appendix/tonsil		

Diagnostic Approach CD10 (neprilysin) is a zinc-dependent cell membrane metalloprotease involved in the post-secretory processing of neuropeptides and *vasoactive peptides*. Despite the name of CD10 as the common acute lymphoblastic leukemia antigen (CALLA), CD10 is not a cell line- or tumor-specific marker as it is expressed in a long list of tissue and tumor types of lymphoid, epithelial, and mesenchymal origin mentioned in the above table [6, 7]. In diagnostic immunohistochemistry, CD10 must be used in a panel with other tissue- and cell-specific markers [8]. The expression pattern of CD10 (membranous or cytoplasmic) is highly variable, depending on tumors type but also grade as the cytoplasmic stain is usually seen in poorly differentiated carcinomas.

CD5		
Expression pattern: membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Mantle cell lymphoma	B-CLL, T-ALL, T-cell lymphoma, prolymphocytic leukemia, adenocarcinomas of different origin, atypical thymoma, and thymic carcinoma	T cells, subset of B cells of mantle zone of the spleen and lymph nodes
Positive control: appendix/tonsil		

Diagnostic Approach CD5 (lymphocyte antigen T1, Leu-1) is a glycoprotein receptor expressed in the majority of T-lymphocytes and subset of B-lymphocytes including mantel zone lymphocytes. CD5 labels different T-cell neoplasms such as T-ALL, adult and peripheral T-cell lymphoma, mycosis fungoides, and T-cell large granular lymphocytic leukemia. The expression of CD5 is not restricted to T-lymphocytes but also found in a small subset of B-lymphocytes and lymphomas of B-cell origin mainly mantle cell lymphoma and B-CLL (Figs. 16.1 and 16.2).

Diagnostic Pitfalls The expression of CD5 is not limited to lymphoid tissue but found in adenocarcinomas of different origin, renal cell carcinoma, and adrenocortical carcinoma in addition to squamous cell carcinoma. Furthermore, CD5 is a diagnostic marker for atypical thymoma and thymic carcinoma; a focal weak expression of CD5 can be also found in mesothelioma, transitional carcinoma, squamous cell carcinoma, and adenocarcinomas of different origin [9].

Ki-67: Ki-67 is a nonhistone nuclear protein involved in the early steps of polymerase I-dependent ribosomal RNA synthesis and DNA replication expressed in active cell cycles. The expression of Ki-67 begins in the G_1 phase and persists during the active phases of cell cycle throughout the S, G_2, and M phases, whereas the peak of the Ki-67 expression appears in the early M phase. Ki-67 is rapidly catabolized at the end of the M phase with a half-life of 1–1.5 h and is undetectable in the G_0 phase or in the initial stage of the G_1 phase. Cells during the DNA repair also lack the Ki-67 expression.

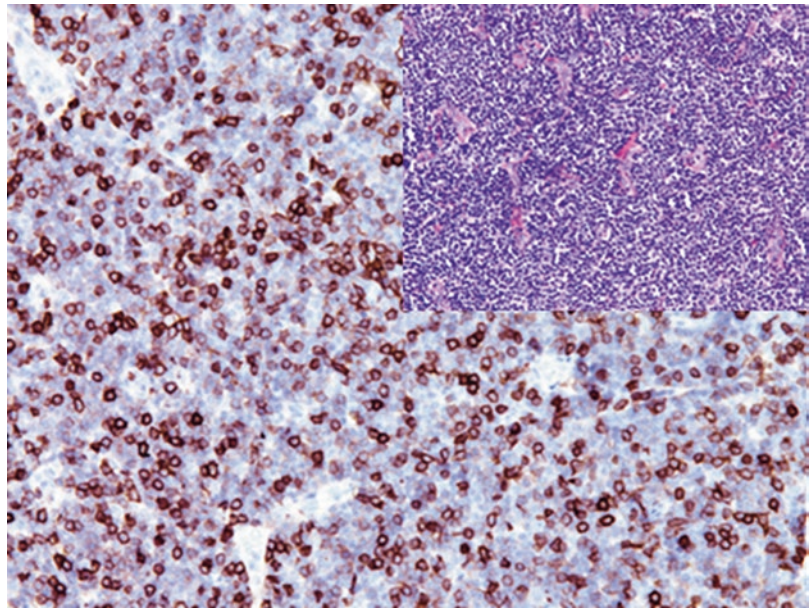


Fig. 16.1 Weak to moderate CD5 expression in cells of B-CLL. T-lymphocytes with strong CD5 expression

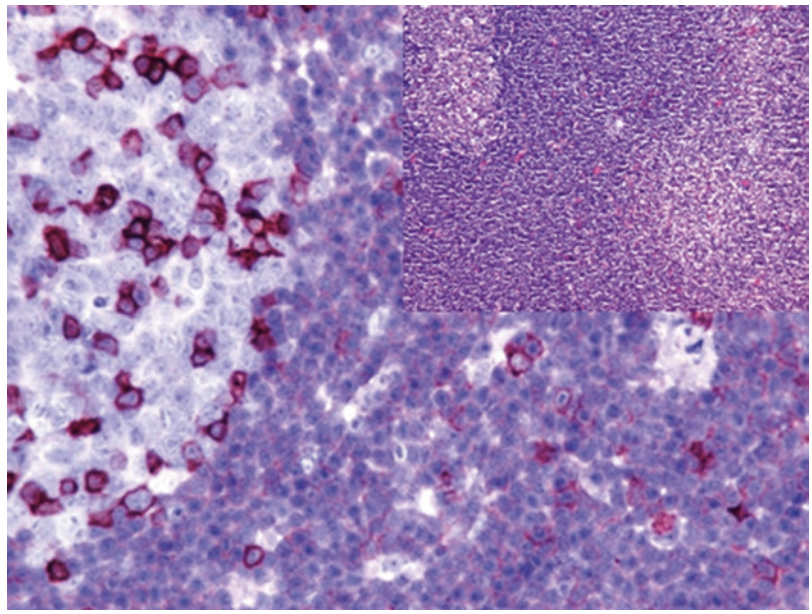


Fig. 16.2 Cells of mantel cell lymphoma showing moderate membranous CD5 expression. T-lymphocytes with strong CD5 expression

The expression of Ki-67 strongly correlates with the intensity of cell proliferation and tumor grade. In routine histopathology, Ki-67 is an important marker for the assessment of cell proliferation. The Ki-67 index is an important criterion for tumor diagnosis (benign, borderline, malignant, low- or high-grade tumor). Furthermore, it is a helpful marker to differentiate between atrophy or thermal alterations and dysplasia (Fig. 16.3). Few tumors show a Ki-67 index of nearly 100%, which can be used as a diagnostic clue; most representative examples are small cell lung carcinoma, Burkitt lymphoma, and plasmablastic lymphoma (Fig. 16.4). In routine hematopathology, the Ki-67 index is an important parameter to classify low and high malignant

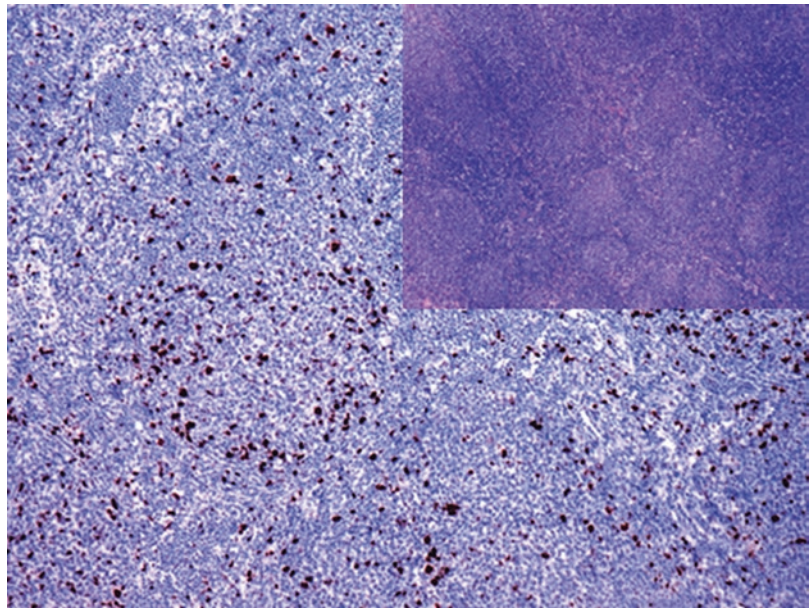


Fig. 16.3 Characteristic low proliferation index of neoplastic follicles in grade 1–2 follicular lymphoma

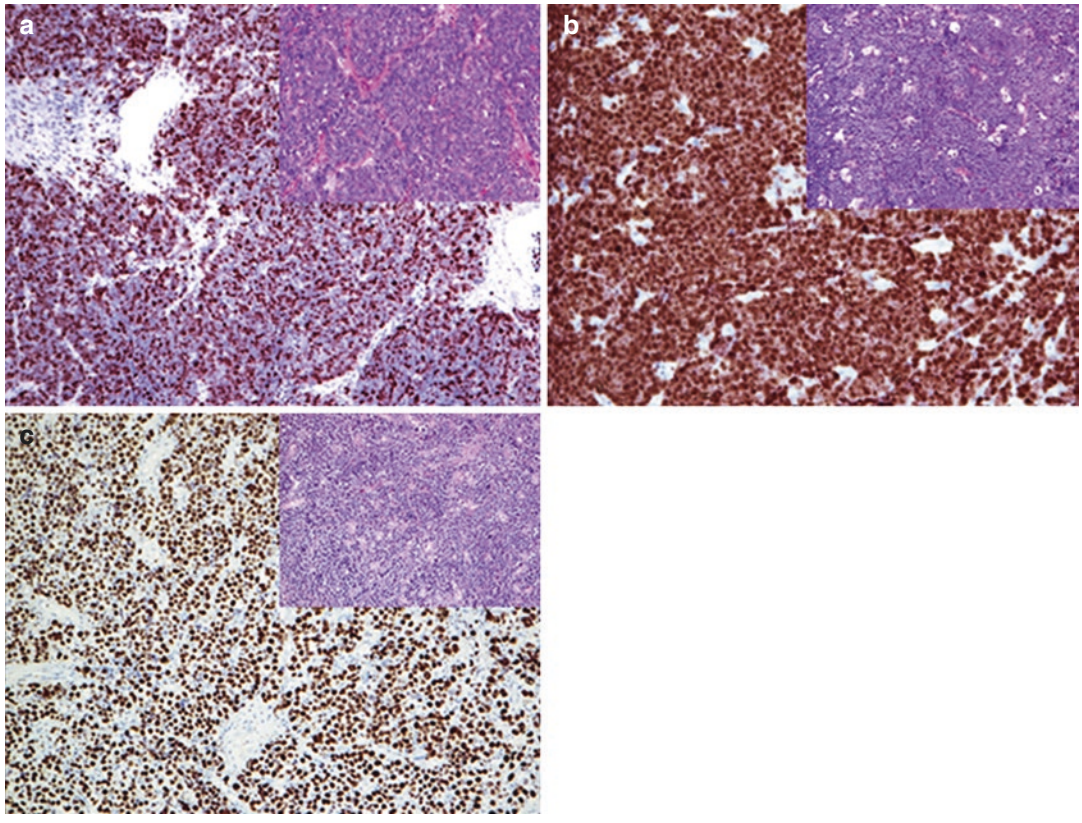


Fig. 16.4 Three tumor types with high Ki-67 index (~100%): (**a**) small cell carcinoma, (**b**) Burkett's lymphoma, and (**c**) plasmablastic lymphoma

lymphomas. Additionally, the Ki-67 index is a well-known prognostic marker correlating with the biological behavior of tumors such as breast carcinoma and neuroendocrine tumors. Nonetheless, it is a challenge to standardize Ki-67 staining and to establish a robust and reliable Ki-67 evaluation, which tends to show a considerable interlaboratory variability. This markedly hampers its clinical utility.

16.2 Markers and Immunoprofile of B-Cell Neoplasms

Immunohistochemical Markers for B-Cell Lymphoma CD5, CD10, CD19, CD20, CD23, CD79a, PAX-5, bcl-2, bcl-6, cyclin D1, Sox-11, ARTA1, and TdT [2, 3, 8, 10].

CD19 (B4)		
Expression pattern: membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
B-cell lymphoma/ leukemia	AML (M0), blast phase of CML	B cells, follicular dendritic cells
Positive control: appendix/tonsil		

Diagnostic Approach CD19 is a single chain glycoprotein and a member of the immunoglobulin family. CD19 is an early naïve B-lymphocyte antigen, which remains through the B-lymphocyte differentiation stages and disappears in the plasma cell stage. It is also expressed on the surface of follicular dendritic cells. CD19 is an excellent B-lymphocyte marker, and antibodies to CD19 are available for both flow cytometry and paraffin histology [11].

CD20 (B1 antigen)		
Expression pattern: membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
B-cell lymphoma/ leukemia		B cells, follicular dendritic cells
Positive control: appendix/tonsil		

Diagnostic Approach CD20 is a transmembrane non-glycosylated phosphoprotein acting as receptor during B-cell activation and differentiation. CD20 is expressed in B cells after CD19 in the naïve B-lymphocytes and remains until late stages of B-lymphocyte differentiation but disappears in the plasma cell stage.

Diagnostic Pitfalls CD20 is a pan-B-lymphocyte marker, but some types of B-cell lymphomas are CD20 negative or show a very weak expression level; consequently in doubtful cases, it is important to use two B-cell markers to assure or exclude the B-cell origin of the neoplasm. Optimal combinations are CD20/CD19 and CD20/PAX-5 or CD20/CD79. Generally, the expression of CD20 is restricted to B-lymphocytes, but rare cases of CD20 expression in peripheral T-cell lymphoma are reported. Another diagnostic pitfall is the interpretation of CD20 stain in patients after the specific CD20 immunotherapy (rituximab). Nuclear or nucleolar CD20 staining pattern are nonspecific.

CD23 (low-affinity IgE receptor)		
Expression pattern: membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
B-CLL, follicular dendritic cell tumors	Mediastinal large B-cell lymphoma, lymphoplasmacytic lymphoma, hairy cell leukemia, DLBCL	Follicular dendritic cells, EBV-transformed lymphoblasts, monocytes, platelets
Positive control: appendix/tonsil		

Diagnostic Approach CD23, also known as low-affinity IgE receptor, is a type II transmembrane glycoprotein involved in the regulation of IgE response. CD23 is expressed on mature B-lymphocytes, follicular dendritic cells, and activated macrophages. CD23 is an essential marker used to discriminate B-CLL from other lymphoma types with similar morphology (Fig. 16.5). CD23 also labels mediastinal large B-cell lymphoma and lymphoplasmacytic lymphoma. It is also an important marker for follicular dendritic cell tumors.

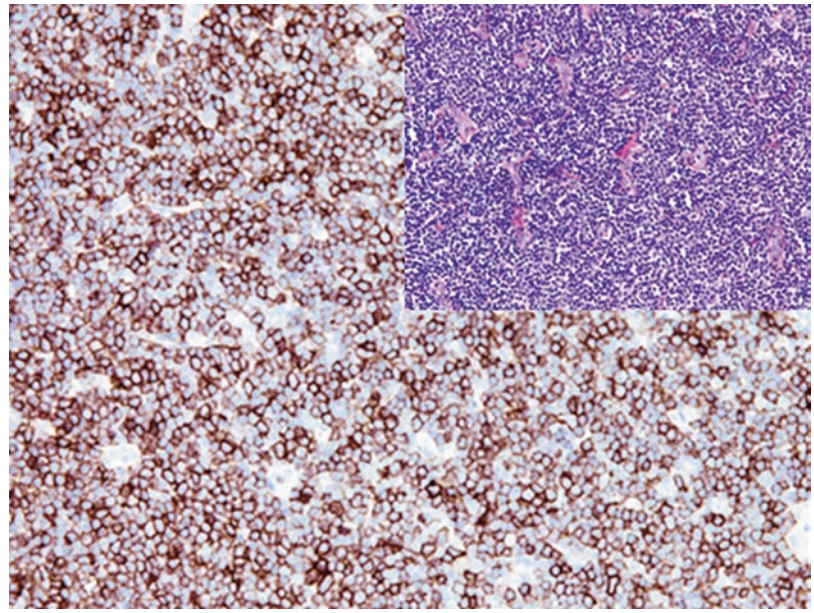


Fig. 16.5 Membranous CD23 expression in B-CLL

CD79a		
Expression pattern: membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
B-cell leukemia/ lymphomas	Acute promyelocytic leukemia (FAB-M3), multiple myeloma	B cells, small population of CD3+ T cells, subset of endothelial cells
Positive control: appendix/tonsil		

Diagnostic Approach CD79a is a disulfide-linked heterodimer associated with the membrane-bound immunoglobulin; it appears in the pre-B-lymphocyte stage and persists until the plasma cell development, rendering the majority of normal and neoplastic plasma cells positive for CD79a. CD79a exhibits a membranous stain, but plasma cells may also show a cytoplasmic staining pattern. The expression of CD79a is independent of the expression of CD20 and remains positive after the anti-CD20 immunotherapy.

Diagnostic Pitfalls CD79a is less reliable than CD20 for the diagnosis of B-cell lymphoma, as it is positive in a small fraction of T-ALL, AML (FAB-M3), and the majority of plasma cell neoplasms (see above).

PAX-5 (B-cell-specific activator protein, BSAP)		
Expression pattern: nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
B-cell lymphoma/ leukemia, Reed-Sternberg cells of classic Hodgkin's lymphoma	Merkel cell carcinoma, alveolar rhabdomyosarcoma, small cell carcinoma, Wilms' tumor, glioblastoma and neuroblastoma, mesonephric and Müllerian tumors	Pre-B to mature B cells
Positive control: appendix/tonsil		

Diagnostic Approach PAX-5 is a member of the PAX (**pa**ired bo**x**) family of transcription factors involved in tissue and organ differentiation. PAX-5 (also known as B-cell activator protein) is a B-cell-specific transcription factor encoded by the gene located at 9p13 and expressed in the early pro-B, pre-B, and naïve stages of B-cell development until the mature B cells [12]. The PAX-5 gene is involved in the t(9;14)(p13;q32) translocation associated with the plasmacytoid subtype of small lymphocytic lymphoma. PAX-5

is also expressed in the L&H cells of nodular lymphocyte-predominant Hodgkin's lymphoma. T-lymphocytes, plasma cells, and macrophages are constantly PAX-5 negative.

Diagnostic Pitfalls PAX-5 can be positive in some tumors resembling lymphoma such as Merkel cell carcinoma and small cell carcinoma and also rarely in acute lymphoblastic lymphoma of T-cell origin [13, 14]. PAX-5 maybe also expressed in acute myeloid leukemia, mainly the type associated with the t(8;21)(q22;q22) translocation. PAX-5 positivity is reported in rare cases of breast, endometrial, and transitional carcinomas in addition to alveolar rhabdomyosarcoma, but it is constantly negative in embryonal-type rhabdomyosarcoma [15, 16].

Cyclin D1 (bcl-1)		
Expression pattern: nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Mantle cell lymphoma	Inflammatory pseudotumor (myofibroblastic tumor), hairy cell leukemia, multiple myeloma, parathyroid adenoma/carcinoma, pulmonary adenocarcinoma, breast and prostate carcinoma, transitional cell carcinoma	Cells in the G_1 phase of cell cycle, histiocyts, endothelial cells
Positive control: mantle cell lymphoma		

Diagnostic Approach Cyclin D1 (also known as bcl-1) is a cell cycle protein involved in the regulation of cyclin-dependent kinases of the first gap phase (G_1) of the cell cycle. The expression of cyclin D1 is not restricted to lymphoid neoplasms and found in a number of nonlymphoid epithelial and mesenchymal tumors. The cyclin D1 overexpression—caused by the t(11;14) translocation associated with mantle cell lymphoma—makes it a characteristic marker for this lymphoma type (Fig. 16.6). In routine immunohistochemistry, cyclin D1 is usually used in combination with CD5, Sox-11, and other B-cell markers [8, 17].

A subset of multiple myeloma harbors also the t(11;14) translocation and is positive for cyclin D1; this myeloma type is usually associated with favorable prognosis.

Diagnostic Pitfalls Other lymphoma types exhibiting similar morphology such as hairy cell leukemia and B-CLL may be also positive for cyclin D1; however, the stain intensity is much less than that of mantle cell lymphoma [18]. A small subset of mantle cell lymphoma lacks the expression of cyclin D1; this subset is usually positive for Sox-11, which to consider in the differential diagnosis.

Sox-11		
Expression pattern: nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Mantle cell lymphoma	Hairy cell leukemia, Burkitt lymphoma, T- and B-ALL, prolymphocytic leukemia, ovarian carcinoma	Immature neurons
Positive control: mantel cell lymphoma		

Diagnostic Approach Sox-11 is a member of the Sox family of transcription factors (**s**ex-determining region Y-b**ox 11**), a transcription factor involved in embryogenesis and development of the central nervous system. Sox-11 strongly stains both cyclin D1 positive and negative mantle cell lymphoma (Fig. 16.7) in addition to other lymphoma types including hairy cell leukemia and ALL [19–21].

Sox-11 stains also a subset of ovarian carcinomas, generally associated with good prognosis.

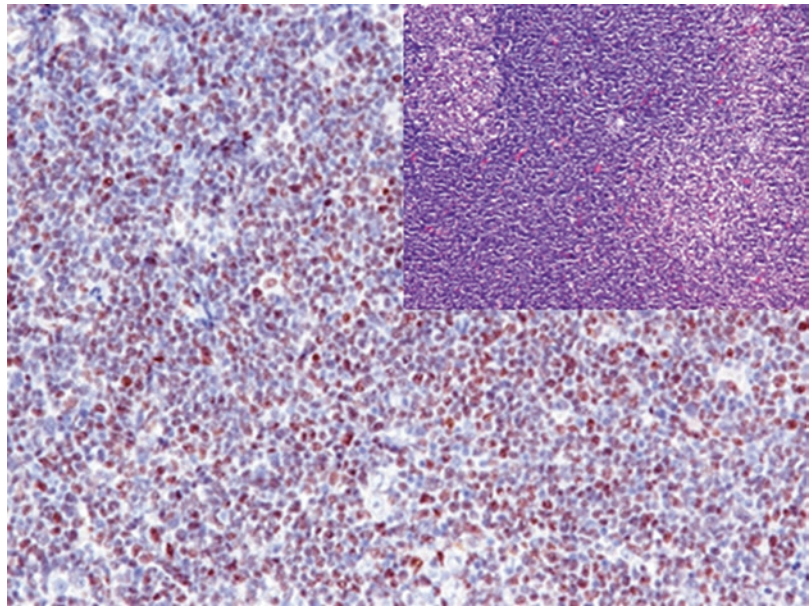


Fig. 16.6 Strong nuclear cyclin D1 expression in mantel cell lymphoma

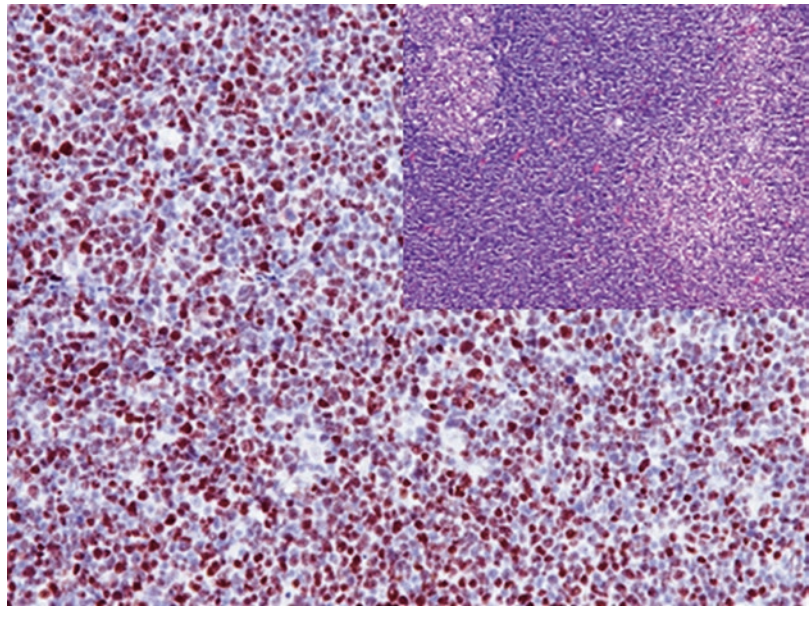


Fig. 16.7 Strong nuclear Sox-11 expression in mantel cell lymphoma

bcl-6		
Expression pattern: nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Follicular lymphoma (intra- and interfollicular cells), anaplastic CD30+ large cell lymphoma	Burkitt lymphoma, diffuse large B-cell lymphoma, mediastinal large B-cell lymphoma, L&H cells in nodular lymphocyte-predominant Hodgkin's lymphoma, ALK + anaplastic large cell lymphoma, angioimmunoblastic lymphoma, T-ALL	Germinal centers of lymph nodes, subset of intrafollicular CD4+ T-lymphocytes
Positive control: appendix/tonsil		

Diagnostic Approach bcl-6 (**B-c**ell **l**ymphoma **6** protein) is a sequence-specific transcriptional repressor protein expressed in normal germinal center B-lymphocytes with high proliferation rate and active somatic mutations. bcl-6 is a marker for lymphomas of germinal center origin such as follicular lymphoma (intra- and interfollicular cells), Burkett's lymphoma, majority of Hodgkin cells, and nodular lymphocyte-predominant Hodgkin's lymphoma [8]. Mutations within the bcl-6 gene are found in about 40% of diffuse large B-cell lymphoma and 15% of follicular lymphoma causing the overexpression of bcl-6 [22]. bcl-6 is also found in some NK-/T-cell lymphoma types such as angioimmunoblastic lymphoma and T-ALL. Mantle cell lymphoma, marginal zone lymphoma, and ALL are constantly bcl-6 negative.

bcl-2		
Expression pattern: cytoplasmic (mitochondrial membrane)		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Follicular lymphoma	Majority of B-cell lymphomas, subset of T-cell lymphoma, basal cell carcinoma, adrenocortical tumors, solitary fibrous tumor, synovial sarcoma, hemangiosarcoma, neurofibroma, schwannoma, nasopharyngeal carcinoma, dermatofibrosarcoma protuberans, spindle cell lipoma, rhabdomyosarcoma	Small B-lymphocytes in primary follicles and in the mantle and marginal zones, subset of T-lymphocytes, medullary cells in thymus, adrenal cortex, basal keratinocytes of the epidermis
Positive control: appendix/tonsil		

Diagnostic Approach bcl-2 (**B-c**ell **l**ymphoma **2** protein) is a family of regulator proteins involved in the regulation of programmed cell death divided into two main groups: the bcl-2 group as antiapoptotic and proapoptotic group (effectors and activators). The bcl-2 proteins are encoded by the bcl-2 gene on chromosome 18q21. The bcl-2 gene is transcribed into three mRNA variants, which are translated into two homologous integral cell and mitochondrial membrane proteins.

The t(14;18)(q32;q21) translocation characteristic for 90% follicular lymphoma juxtapose the bcl-2 gene to the Ig heavy chain gene resulting the deregulation of the bcl-2 gene and the overexpression of the bcl-2 protein giving a survival advantage for the lymphoma cells. One of the main diagnostic benefits of bcl-2 is to distinguish between reactive lymph nodes with follicular hyperplasia exhibiting bcl-2-negative germinal centers and grade 1 follicular lymphoma with bcl-2-positive neoplastic B cells in the follicles (Fig. 16.8) [8]. The bcl-2 expression is found in the majority of B-cell lymphomas and in a subset of T-cell lymphomas. It is also found in a large number of epithelial and mesenchymal tumors [8].

CD11c		
Expression pattern: membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Hairy cell leukemia	AML (M4 and M5), follicular lymphoma, Langerhans cell histiocytosis, lymphoplasmacytic lymphoma, B-CLL, splenic lymphoma, NK lymphoma	Myeloid hematopoietic cells, granulocytes, macrophages, NK cells, dendritic cells, subset of activated T-lymphocytes, histiocytes
Positive control:		

Diagnostic Approach CD11c (also known as integrin alpha X, CR4, LeuM5) is an integrin glycoprotein composed of alpha and beta chains involved in the adhesion and chemotaxis of monocytes, primarily expressed on myeloid hematopoietic cells. CD11c is a marker for different lymphoid and myeloid neoplasms. It is strongly expressed in hairy cell leukemia and

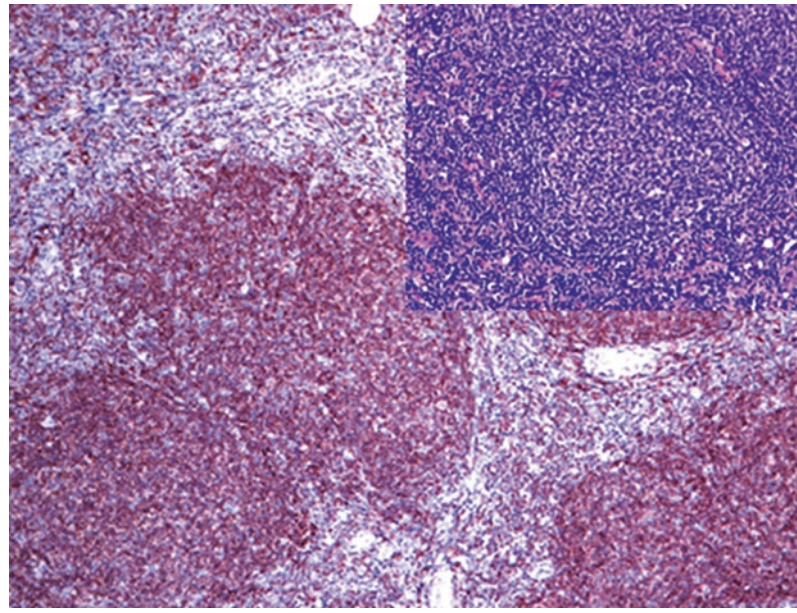


Fig. 16.8 Follicular lymphoma with strong diffuse bcl-2 expression in neoplastic follicles

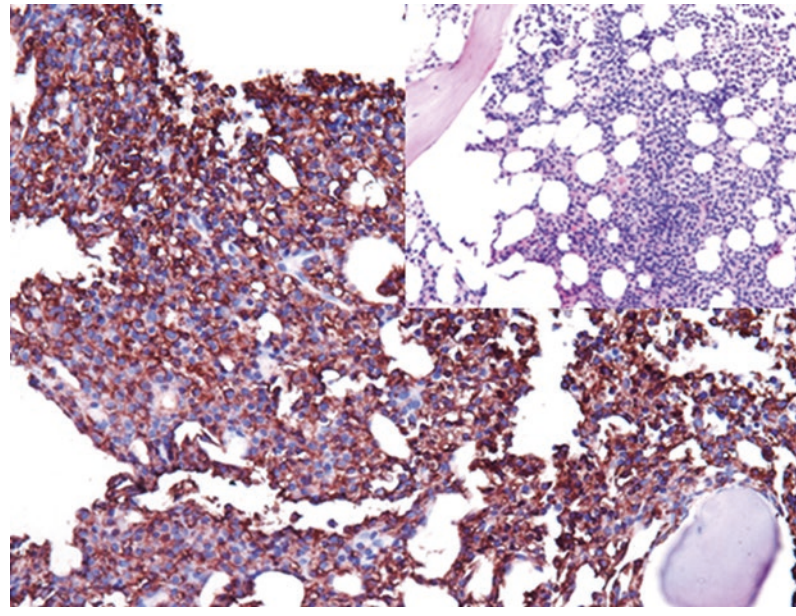


Fig. 16.9 Hairy cell leukemia, with CD11c-positive leukemia cells in the bone marrow

natural killer cell lymphoma (Fig. 16.9). CD11c is also found in about 50% of AML (M4 and M5) and in some cases of follicular lymphoma, Langerhans cell histiocytosis, lymphoplasmacytic lymphoma, splenic lymphoma with villous lymphocytes, and B-CLL. The expression of CD11c on B-CLL cells is usually associated with good prognosis.

Tartrate-resistant acid phosphatase (TRAP)		
Expression pattern: cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Hairy cell leukemia, osteoclastoma (giant cell tumor)	Mantel cell lymphoma, mediastinal B-cell lymphoma, splenic marginal cell lymphoma	Osteoclasts, macrophages, lymphocytes of the marginal zone, neurons, decidual cells, prostatic glands, red blood cells
Positive control: osteoclasts, hairy cell leukemia		

Diagnostic Approach Tartrate-resistant acid phosphatase (TRAP) is a glycosylated iron-binding metalloprotein enzyme found in different tissue types and is highly expressed in osteoclasts and macrophages. TRAP is specific marker for hairy cell leukemia but should be used in combination with other markers such as CD11c and DBA.44 (Fig. 16.10) [23].

Diagnostic Pitfalls Other lymphoma type such as marginal zone B-cell lymphoma may reveal weak TRAP positivity. TRAP is also expressed in bone marrow marcophages.

Immunoglobulin Superfamily Receptor Translocation-1: IRTA-1 is a cell surface receptor involved in the lymphogenesis of B-lymphocytes in addition to intercellular communication. IRTA-1 is helpful marker to decimate between marginal zone lymphoma and other lymphoma types as it is expressed in more than 90% of extranodal marginal zone lymphoma and in about 75% of nodal marginal zone lymphoma but negative in splenic marginal zone lymphoma. Other lymphoma types including B-CLL, mantel cell lymphoma, follicular lymphoma, Burkitt lymphoma, hairy cell leukemia, and plasma cell neoplasms also lack the expression of IRTA-1 [24, 25]. IRTA-1 cannot distinguish between reactive and neoplastic marginal zone lymphocytes.

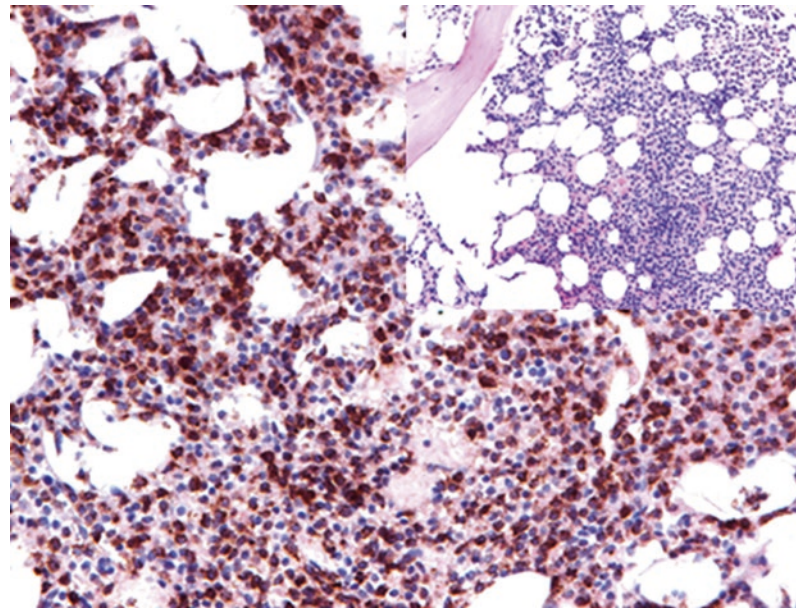


Fig. 16.10 Bone marrow trephine infiltrated by cells of hairy cell leukemia exhibiting strong cytoplasmic TRAP expression

LIM-only transcription factor 2 (LMO2)		
Expression pattern: nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Follicular lymphoma, mediastinal large B-cell lymphoma, Burkitt lymphoma	B- and T-ALL, endothelial tumors. GIST, myoepithelial tumors, juvenile xanthogranuloma	Germinal centers of lymph nodes, hematopoietic precursors, endothelium, breast myoepithelial cells, basal cells of prostatic gland, endometrial glands in secretory phase
Positive control: tonsil/lymph node		

LIM-Only Transcription Factor 2 LMO2 (also known as TTG2 or RBTN2) is a transcription factor regulating the yolk sac angiogenesis and erythropoiesis, normally expressed in erythroid and myeloid precursors as well as megakaryocytes and endothelial cells. The LMO2 protein is expressed in B-lymphocytes of germinal centers. LMO2 is a marker for several lymphoma types derived from germinal center cells. It is expressed in up to 70% of all grades of follicular lymphoma, mediastinal large B-cell lymphoma, Burkitt lymphoma and diffuse large B-cell lymphoma, and B- and T-ALL. CLL, mantel cell lymphoma, marginal zone lymphoma, lymphoplasmacytic lymphoma, and peripheral T-cell lymphomas usually lack the expression of LMO2. LMO2 is expressed in lymphocyte-predominant Hodgkin's lymphoma but not in classical Hodgkin's lymphoma. Furthermore LMO2 labels the myeloid blasts of acute myeloid leukemia [26, 27]. In addition to lymphoid and hematopoietic neoplasms, LMO2 labels normal endothelium of blood and lymph vessels and the majority of benign and malignant endothelial tumors [28].

Human Germinal Center-Associated Lymphoma HGAL: also known as germinal center B-cell-expressed transcript 2 (GCET-2) is exclusively expressed in the cytoplasm and on the membrane of germinal center B-lymphocytes and specially accentuated in the proliferating cells within the dark zone of germinal centers. HGAL is involved in the regulation of lymphocyte motility. Lymphocytes within the mantle and marginal zones as well as interfollicular and paracortical regions lack the expression of HGAL. HGAL is a marker for B-cell lymphomas derived from germinal center lymphocytes and expressed in 100% of Burkitt lymphoma, more than 90% of follicular lymphomas and mediastinal lymphoma, and about 70% of diffuse large B-cell lymphoma. The expression of HGLA is reported in less than 5% of marginal zone lymphoma whereas mantel cell lymphoma and B-CLL completely negative for HGAL [29, 30].

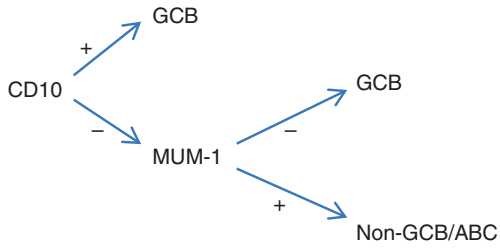
Lymphoid Enhancer-Binding Factor LEF-1: is a nuclear protein and a member of the T-cell-specific factor family that binds to the T-cell receptor playing a role in the regulation of cell proliferation and lymphopoieses. LEF-1 is normally expressed in pre-B- and T-lymphocytes but not in mature B cells. In lymphomas, LEF-1 labels the neoplastic small lymphocytes of chronic lymphocytic leukemia (CLL) but negative in other small B-cell lymphomas [31]. It is also found in about one third of diffuse large B-cell lymphoma. LEF-1 is not a specific lymphoma marker as it is also expressed in different carcinoma types such as colorectal adenocarcinoma [32].

Immunoprofile of B-cell neoplasms				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
Precursor B-lymphoblastic leukemia/lymphoma	*TdT*, HLA-DR (CD74), *CD19*, CD79a, PAX-5 Proliferation index (Ki-67): 50–80%	*CD10*[a], CD22, CD24, CD45, CD99, CD34, FLI-1, LMO2	CD20, CD13	
B-cell chronic lymphocytic lymphoma (B-CLL)/small lymphocytic lymphoma	*CD5*, CD19, *CD20*, CD22, *CD23*, CD74, CD79a, *CD160*, *CD200*, *LEF-1*, PAX-5, p27, bcl-2, sIgM Proliferation index (Ki-67): ~ 5%	CD22, CD43, MUM-1, sIgD	CD11c, CD38[b]	CD10, Sox-11, bcl-6
Monoclonal B-cell lymphocytosis	See B-CLL immunoprofile (B-cell account in peripheral blood <5 x10 [9]/L with B-CLL phenotype with no signs of lymph node involvement)			
B-cell prolymphocytic leukemia	*CD19*, *CD20*, CD22, CD25, CD27, CD74, CD79a, PAX-5, bcl-2	sIgM, sIgD	CD5	CD10, CD23, CD43, CD138, cyclin D1
Lymphoplasmacytic lymphoma	*CD19*, *CD20*, CD22, CD43, CD74, CD79a, *CD200*, PAX-5, IgM Proliferation index (Ki-67): ~5–10%	CD38, CD138, MUM-1, bcl-2, MYD88	CD5	CD10, CD23, cyclin D1
Mantle cell lymphoma	*CD5*, CD19, CD20, CD22, CD37, CD43, CD74, CD79a, sIgM, sIgD, *cyclin D1*, *Sox-11*, PAX-5, FMC-7 Proliferation index (Ki-67): 5–50%	bcl-2		CD10, CD11c, CD23, bcl-6
Follicular lymphoma/in situ follicular neoplasia/ duodenal-type follicular lymphoma	CD19, *CD20*, CD22, CD74, CD79a, PAX-5, *HGAL*, sIg, bcl-2 Nodular meshwork of follicular dendritic cells positive for CD21 and CD23 *Proliferation index (Ki-67) in bcl-2-positive neoplastic follicles: < 20%* *Proliferation index (Ki-67) in bcl-2-negative reactive follicles: > 60%*	CD10, *bcl-6*, bcl-2 (in grade 3 follicular lymphoma), *LMO2* κ/λ light chain restriction	bcl-2 (in primary cutaneous follicular lymphoma)	CD5, CD23, CD43, Sox-11, cyclin D1
Pediatric-type follicular lymphoma	CD19, CD20, CD22, CD74, CD79a, PAX-5, CD10, *HGAL*, *LMO2*, sIg, Proliferation index (Ki-67): >30%	*bcl-6*, CD43		MUM-1, *bcl-2*[c]
Large B-cell lymphoma with IRF-4 rearrangement	CD19, CD20, CD22, *MUM-1*, *bcl-6*	CD10, bcl-2	CD5	
Primary cutaneous follicle center lymphoma	CD20, PAX-5, bcl-6	CD10, bcl-2	CD30, CD23	CD3, CD5, CD43, cyclinD1
Nodal marginal zone B-cell lymphoma	CD19, *CD20*, CD21, CD22, CD35, CD74, CD79a, PAX-5, sIgM	sIgA, sIgG, CD11c, bcl-2, *IRTA-1*	CD43, CD38, MUM-1, TRAP	sIgD, CD5, CD10, CD 23, bcl-6, Sox-11, cyclin D1
Extranodal marginal zone B-cell lymphoma of MALT type	CD19, *CD20*, CD21, CD22, CD35, CD74, CD79a, PAX-5, sIgM, *IRTA-1*	CD11c, MUM-1, sIgD, sIgA, sIgG, bcl-2	CD43	CD5, CD10, CD23, Sox-11, cyclin D1, bcl-6
Splenic marginal zone B-cell lymphoma	CD19, *CD20*, CD21, CD22, CD35, CD74, CD79a, PAX-5, bcl-2, sIgM, sIgD, Proliferation index (Ki-67): < 5%	sIgA, sIgG, CD11c	CD5	CD43, CD10, CD23, CD25, CD43, CD103, bcl-6, *cyclin D1*, annexin A1, IRTA-1

Immunoprofile of B-cell neoplasms				
Hairy cell leukemia	*CD11c*, CD19, *CD20*, CD22, CD25, CD74, CD79a, *CD103*, CD123, *annexin A1*, *TRAP*, *DBA.44*, *BRAF^{V600E}*, PAX-5, cyclin D1, sIgM, FMC7 Proliferation index (Ki-67): <5%	CD23, CD68 (cytoplasmic dots), PCA-1, HC1, HC2	CD5	*CD10*, *CD23*, *CD43*, bcl-6
Diffuse large B-cell lymphoma (DLBCL) - Germinal center cell type (GCB)[d] - Activated B-cell type (ABC)[d]	CD19, *CD20*, CD22, CD74, CD79a, CD45, PAX-5 Proliferation index (Ki-67): > 40%	bcl-6	bcl-2, CD5, CD30, fascin, MUM-1[e]	CD3, CD15, CD200
Primary cutaneous diffuse large B-cell lymphoma, leg type	CD20, CD70a, PAX-5, bcl-2, MUM-1			CD10
T-cell-/histiocyte-rich variant of diffuse large B-cell lymphoma	Neoplastic cells: CD19, *CD20*, CD22, CD74, CD79a, CD45, PAX-5, bcl-6, BOB 1, OCT-2 Nonneoplastic cells (>80% of cell population): positive for CD3, CD8, cytotoxic molecules, and CD68 in histiocytes		CD30, EMA	CD3, CD15, bcl-2, PU.1
Mediastinal (thymic) large B-cell lymphoma	CD19, *CD20*, CD45, CD74, CD79a, CD200, PAX-5	*CD23*, MUM-1, *CD30, HGAL, LMO2*	CD10	CD5, CD21
ALK-positive large B-cell lymphoma	*ALK*, EMA, CD138, VS38c	CD4, κ or λ Ig light chains	CD45, CD79a	CD3, CD20, CD30,
Plasmablastic lymphoma	CD38, CD138, VS38c, MUM-1, EBV (EBER), LCA Proliferation index (Ki-67): > 90%	CD79a, EMA, CD10	CD30	CD20, PAX-5, CD56
Intravascular large B-cell lymphoma	CD20, CD79a, PAX-5	Prostatic acid phosphatase	CD5, CD10	
Primary effusion lymphoma	CD45, *CD79a*, CD38, CD138, VS38c, *PAX-5*, *HHV-8*, MUM-1	CD30, EBV	CD20, CD19	CD43, bcl-6
Burkitt lymphoma	*CD10*, CD19, *CD20*, CD22, CD74, CD79a, PAX-5, sIgM, *c-myc*, *HGAL*, CD43, p53 Proliferation index (Ki-67): > 95%	bcl-6, EBV, LMO2, adipophilin		CD5, CD23, TdT, bcl-2
Burkitt-like lymphoma with 11q aberration	CD19, CD20, CD22, CD38, CD74, CD79a, PAX-5 Proliferation index (Ki-67): > 95%	CD43, bcl-6, sIgG, IgM	CD10	*c-myc*, bcl-2
EBV-positive mucocutaneous ulcer EBV-positive DLBCL	*EBV*[f], CD19, *CD30*, MUM-1, PAX-5	CD20, CD15, bcl-2		
Lymphomatoid granulomatosis	EBV, CD19, *CD20*	CD79a	CD30	CD15

[a]Negative in ALL with 11q23 translocation
[b]The expression of CD38 in B-CLL correlates with worse prognosis
[c]Pediatric-type follicular lymphoma lacks the t(14;18) translocation
[d]See modified Hans algorithm below [33]
[e]Positive in ABC (activated B-cell-like) subtype of DLCBL
[f]EBV antigens: EBER, LMP1, and EBNA2

Algorithm 16.1: Modified Hans Algorithm [33]



- GCB: Germinal center B-cell type
- ABC: Activated B-cell type

16.3 Markers and Immunoprofile of Plasma Cell Neoplasms

CD38, CD138, VS38c, MUM-1, CD56, and κ and λ light chains.

CD38		
Expression pattern: membranous/cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Plasma cell neoplasms, plasmablastic lymphoma	Pre-T-ALL, primary effusion lymphoma, subtypes of B-cell lymphoma	Plasma cells, erythroid and myeloid precursors, early B and T cells, NK cells, pancreatic islets, neuronal tissue
Positive control: appendix		

Diagnostic Approach CD38 (also known as ADP-ribosyl cyclase) is a transmembrane glycoprotein expressed in the majority of CD34-positive pluripotent stem cells and in different maturation stages of B- and T-lymphocytes, plasma cells, and myeloid cells [34]. CD38 is commonly used in diagnostic panels of multiple myeloma. CD38 can be expressed on CLL cells and considered being an adverse prognostic factor.

Diagnostic Pitfalls CD38 has a wide expression spectrum and is found in different hematopoietic and non-hematopoietic cells; accordingly, the CD38 expression does not prove the plasma cell origin, and the plasma cell nature must be confirmed by other more specific markers.

CD138 (syndecan-1)		
Expression pattern: membranous/cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Plasma cell tumors (myeloma, plasmacytoma)	Primary effusion lymphoma; multiple carcinomas including thyroid, breast, lung, head and neck, urothelium, prostatic, and liver; neuroendocrine tumors; thymoma; tumors of the adrenal cortex; keratoacanthoma; malignant melanoma; osteoid-forming tumors	B-cell precursors, plasma cells, stratified squamous epithelium, hepatocytes
Positive control: tonsil/squamous epithelium		

Diagnostic Approach CD138 (syndecan-1) is a transmembrane antigen and one of the four members of the syndecan family. The expression of CD138 is found in different maturation stages of B-lymphocytes and plasma cells and in different types of epithelial and mesenchymal cells; nevertheless, CD138 is one of the important markers for plasma cell neoplasms.

Diagnostic Pitfalls CD138 is widely used as a marker for plasma cells and plasma cell neoplasms. However, the expression of CD138 is found in a large number of epithelial tumors and some mesenchymal tumors. Among the epithelial tumors, CD138 is found in squamous cell carcinoma and adenocarcinomas of different origins including pulmonary and prostatic adenocarcinomas, which makes it necessary to consider these carcinomas in the differential diagnosis [35]. A particular pitfall is the plasmacytoid urothelial carcinoma, which

is often strongly CD138 positive and can be mistaken for a plasmacytoma. To differentiate between epithelial and plasma cell tumors, it is recommended to run a parallel reaction with a pan-cytokeratin antibody but not EMA as EMA may be also positive in plasma cell disorders as well [36]. The expression profile of κ and λ light chains is also important to confirm the diagnosis of plasma cell neoplasia and determine the clonality of the plasma cell population. CD138 is also expressed in other mesenchymal tumors such as alveolar soft part sarcoma, synovial sarcoma, and schwannoma in addition to malignant melanoma and bone-forming tumors including osteosarcoma [37].

MUM-1 (multiple myeloma oncogene 1/IRF4)		
Expression pattern: nuclear/cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Plasma cell neoplasms, diffuse large B-cell lymphoma ABC type	Hodgkin's lymphoma, CLL, marginal zone lymphoma, DLBCL, malignant melanoma	B cells (centrocytes), plasma cells, activated T cells
Positive control: appendix		

Diagnostic Approach The MUM-1 protein (**mu**ltiple **m**yeloma **1**) is a lymphocyte-specific transcriptional activator also known as interferon regulatory factor 4 expressed in the final differentiation stage of intra-germinal center B cells. MUM-1 is a marker for post-germinal center B cells, plasma cells, and subset of T cells and related lymphoma types in addition to Hodgkin cells. MUM-1 is usually negative in the cells of nodular lymphocyte-predominant Hodgkin's lymphoma.

Diagnostic Pitfalls MUM-1 stains also a subset of malignant melanoma, which can be also positive for other plasma cell markers such as CD138 and VS38c. Because of the multilineage expression of the MUM-1 protein, the immunostaining results must be carefully interpreted in combination with additional more specific markers to exclude other possible differential diagnoses [38, 39].

VS38c (plasma cell marker)		
Expression pattern: cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Plasma cell neoplasms (myeloma, plasmacytoma), lymphoma with plasmacytic differentiation	Rare carcinoma types of different origin, malignant melanoma, clear cell sarcoma of soft tissue, neuroendocrine tumors	Plasma cells and plasmablasts, B-immunoblasts, epithelial cells (mucous glands, pancreatic epithelium, secretory breast cells, thyroid follicles), melanocytes, osteoblasts
Positive control: appendix		

Diagnostic Approach VS38c (rough endoplasmic reticulum-associated antigen) is a sensitive screening marker for plasma cells and cells with plasmacytoid differentiation. VS38c is expressed on the endoplasmic reticulum in the cell cytoplasm. The expression of VS38c is found in plasma cells, plasmablasts, lymphoplasmacytoid cells, and B-immunoblasts and related neoplasms.

Diagnostic Pitfalls Despite the specificity and high sensitivity of VS38c to normal and neoplastic plasma cell, it is always important to keep in mind that other tumor types such as melanocytic and neuroendocrine tumors may be positive for this marker [40]. Paratrabecular osteoblasts in trephine biopsies are also positive for VS38c.

Kappa and Lambda Light Chains: Each molecule of the five major classes of immunoglobulins is consisted of the combination of two identical heavy chain molecules and two identical light chain molecules. The light chain molecules are divided into two classes, kappa and lambda light chain; on the other hand, each B-lymphocyte

or plasma cell is able to produce either kappa or lambda light chain. In a polyclonal lymphocyte or plasma cell population, the kappa-to-lambda ratio is approximately 2:1. The clonal restriction of one of both chains indicates the monoclonal—neoplastic—nature of the lymphocyte or plasma cell population. In routine histopathology, the expression of the light chains can be indicated either by conventional immunohistochemistry or by in situ hybridization.

Immunoprofile of plasma cell neoplasms				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
Plasma cell myeloma/plasmacytoma – Monoclonal gammopathy of undetermined significance (MGUS) – Heavy chain disease – Plasma cell myeloma – Solitary plasmacytoma of bone – Extraosseous plasmacytoma – Monoclonal immunoglobulin deposition disease	*CD38*, *VS38c*, *CD138*, PCA-1, MUM-1, vimentin *κ or λ Ig light chain restriction* Proliferation index (Ki-67):~50–60%	CD43, CD56, CD79a	CD45, EMA, cyclin D1, Steroid hormone receptors (ER)	CD19, CD20, CD22, PAX-5, E-cadherin

16.4 Markers and Immunoprofile of T-Cell Neoplasms

Immunohistochemical Markers for T-Cell Lymphoma CD2, CD3, CD4, CD7, CD8, CD30, ALK, TCL-1, CXCL13, and TdT [8, 10, 41].

CD2 (LFA-2)		
Expression pattern: membranous/cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
T-cell lymphoma	Neoplastic mast cells (mastocytosis)	Thymocytes, mature peripheral T cells, NK cells
Positive control: appendix/tonsil		

Diagnostic Approach CD2 is a transmembrane glycoprotein (E rosette receptor) that mediates adhesion between T-lymphocytes and other cells. CD2 appears in the early stages of T-cell development. CD2 is an excellent marker for T-lymphocytes and NK cells and labels T-cell lymphomas and the majority of NK neoplasms. CD2 is negative in B-lymphocytes with the exception of a small subset of thymic B cells but negative in all B-cell lymphomas. CD2 is negative in normal mast cells, and the CD2 expression in mast cells is usually a criterion of malignancy.

CD3		
Expression pattern: membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
T-cell lymphomas	NK lymphoma (cytoplasmic stain)	Thymocytes, peripheral T cells, activated NK cells, Purkinje cells of cerebellum
Positive control: appendix/tonsil		

Diagnostic Approach CD3 is a complex structure composed of five polypeptide chains (γ, δ, ε, ζ, and η) forming three dimers. CD3 builds a complex with the T-cell receptor on the membrane of T-lymphocytes responsible for the recognition of antigens leading to the activation of immune response. In the early embryogenesis, CD3 is expressed in the cytoplasm of the prothymocytes and persists through all differentiation stages of T-lymphocytes until mature cells. CD3 is the most common used pan-T-cell marker expressed in the vast majority of T-cell lymphomas. CD3 labels also a subset of the NK lymphomas usually exhibiting a cytoplasmic staining pattern using CD3ε-specific antibody.

CD4		
Expression pattern: membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Mycosis fungoides, T-cell lymphomas	Histiocytic neoplasms, acute myeloid leukemia	Thymocytes, T-helper/T-inducer cells, macrophages, granulocytes, Langerhans cells, dendritic cells, hepatic sinusoidal cells
Positive control: appendix/tonsil		

Diagnostic Approach CD4 is a transmembrane glycoprotein and a member of the immunoglobulin family expressed on the surface of T-helper/T-inducer cells in addition to the majority of thymocytes and a subset of monocytes, macrophages, and dendritic cells. CD4 is a marker of lymphomas originated from these cells, which include the majority of peripheral T-cell lymphomas and cutaneous lymphomas, mainly mycosis fungoides.

Diagnostic Pitfalls In immunohistochemistry and flow cytometry, CD4 must be used in a panel including CD3 and CD8 and CD19. CD4 can be also positive in subtypes of acute myeloid leukemia and histiocytic neoplasms (Fig. 16.11).

CD7		
Expression pattern: membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
T-ALL and T-cell lymphomas	CML, immature myelomonocytic neoplasms, cholangiocarcinoma, pancreas carcinoma	Thymocytes, mature T cells and NK cells, pre-B cells, monocytes, early myeloid cells
Positive control: appendix/tonsil		

Diagnostic Approach CD7 is a membrane-bound protein and a member of the immunoglobulin family involved in T-cell/B-cell interaction. CD7 is expressed in early T-lymphocytes, thymocytes, NK cells, and subset of myeloid cells. The expression of CD7 persists in the majority of mature T-lymphocytes and T cell and NK lymphomas derived from these cells.

Diagnostic Pitfalls CD7 may be positive in a subset of AML and rarely in carcinomas such as pancreatic and bile duct carcinomas [36].

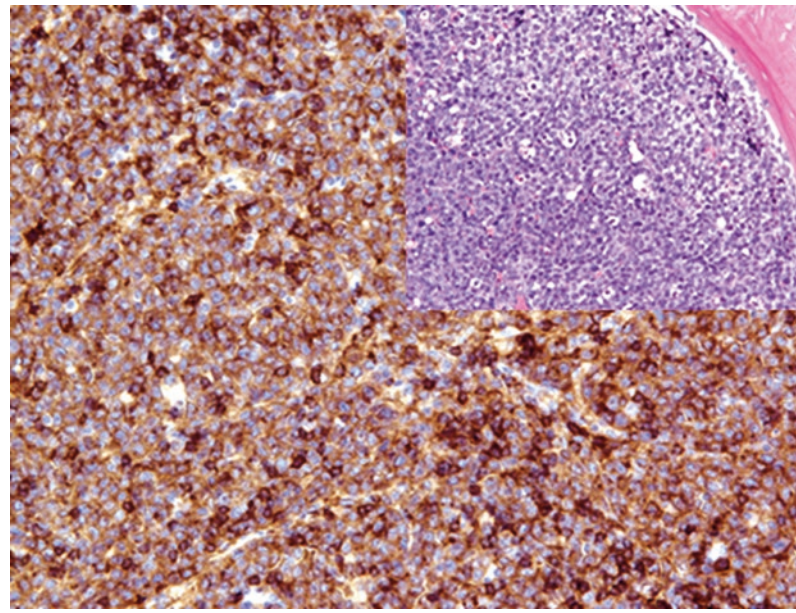


Fig. 16.11 Diffuse CD4 expression in myeloid blasts of AML (M5)

CD8		
Expression pattern: membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Subcutaneous panniculitis-like T-cell lymphoma	T-cell large granular lymphocytic leukemia, CLL, mantle cell lymphoma	Suppressor/ cytotoxic T cells and NK cells
Positive control: appendix/tonsil		

Diagnostic Approach CD8 is a transmembrane glycoprotein functioning as a co-receptor for the T-cell receptor, expressed in the suppressor/cytotoxic T-lymphocytes in addition to a subset of NK cells. CD8 is a marker of many types of T-/NK-cell lymphomas (Fig. 16.12).

Diagnostic Pitfalls CD8 is expressed in a small subset of B-cell lymphomas and generally should be a part of panel with CD3, CD4, and CD20 [36, 42]. The expansion of CD8-positive T-cell population is noted in lymph nodes-associated with acute infectious mononucleosis.

CD30: CD30 (Ki-1) is a transmembrane receptor participating in the regulation of cell transformation, antibody response, and apoptosis. CD30 is normally expressed in activated B, T, and NK cells. In addition to Hodgkin's lymphoma and some other lymphoma types, CD30 is a diagnostic marker for anaplastic large cell lymphoma (Fig. 16.13). CD30 is listed in details in the next chapter.

CD43		
Expression pattern: membranous/cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
T-/NK-cell lymphomas	B-ALL, Burkitt lymphoma, mantle cell lymphoma, marginal zone lymphoma, granulocytic (myeloid) sarcoma, adenoid cystic carcinoma	Activated B cells, T cells, NK cells, plasma cells, granulocytes
Positive control: appendix/tonsil		

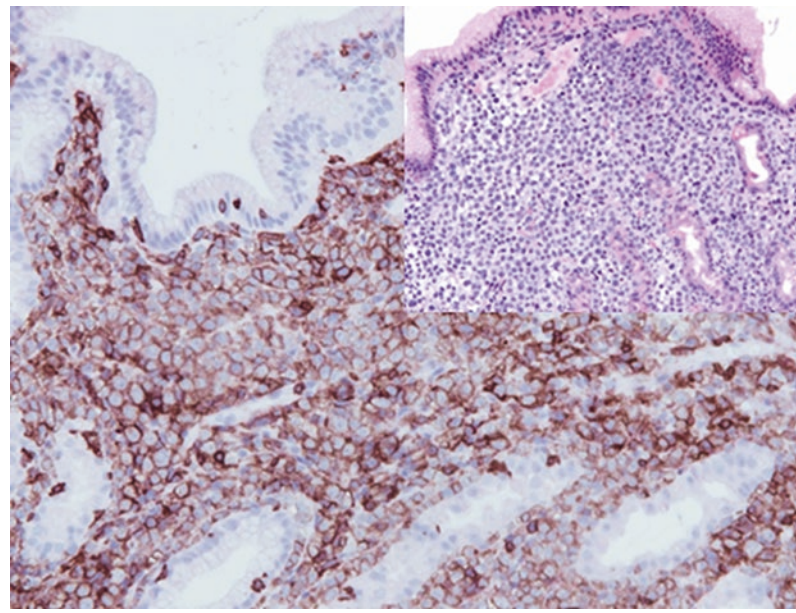


Fig. 16.12 Diffuse CD8 expression in enteropathy-type T-cell lymphoma (type II)

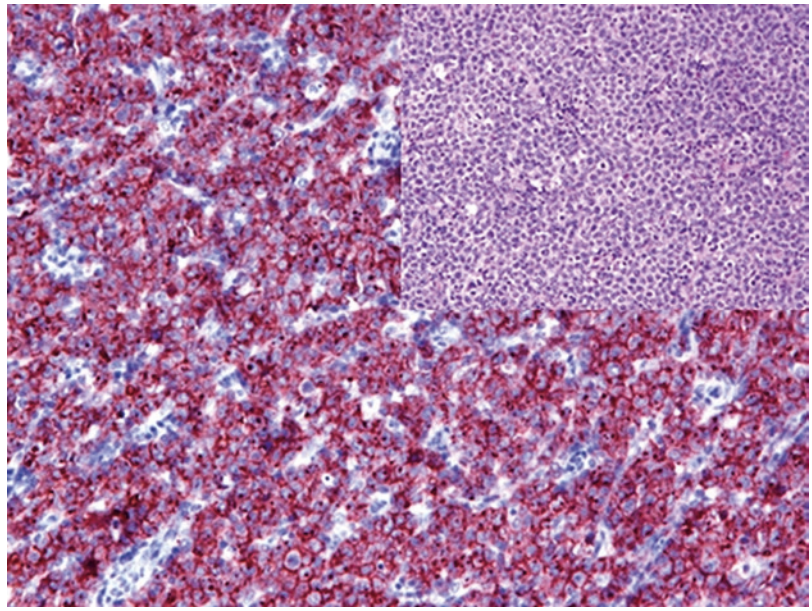


Fig. 16.13 Diffuse CD30 expression in anaplastic large cell lymphoma

Diagnostic Approach CD43 (also known as sialophorin) is expressed on the membrane and in the cytoplasm of the T-/NK-lymphocytes, cells of myeloid lineage, plasma cells, and tumors originating from these cells.

Noteworthy is the so-called "CD43 only pattern" characteristic for some rare tumors that express only CD43 in addition to vimentin. The CD43 only immunophenotype is characteristic for a subset of the following neoplasms, which to consider in the differential diagnosis:

- Myeloid sarcoma and subsets of AML
- Anaplastic large cell lymphoma and NK tumors
- Plasma cell neoplasms
- Langerhans cell histiocytosis

Diagnostic Pitfalls The expression of CD43 correlates with the expression of CD5 and is not restricted to T-cell lymphomas, but also found in many types of B-cell lymphomas such as chronic lymphocytic lymphoma (CLL and SLL), Burkitt lymphoma, mantle cell lymphoma, and nodal/extranodal marginal zone lymphoma [8]. Since normal B-lymphocytes lack the expression of CD43, CD43-positive B-lymphocytes are assumed to be neoplastic. Generally, CD43 must be used in a panel with other more specific lymphoma markers. Adenoid cystic carcinoma is one of the rare non-hematopoietic tumors that express CD43.

Anaplastic lymphoma kinase (ALK, CD246, p80)		
Expression pattern: cytoplasmic/nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Anaplastic large cell lymphoma, inflammatory myofibroblastic tumor	ALK-positive large cell lymphoma, malignant peripheral nerve sheath tumor, rhabdomyosarcoma, neuroblastoma, glioblastoma, Ewing's sarcoma/PNET, leiomyosarcoma, pulmonary non-small cell carcinoma	Glial cells, neurons, endothelial cells, T-lymphocytes
Positive control: anaplastic lymphoma/brain tissue/appendicular ganglion cells		

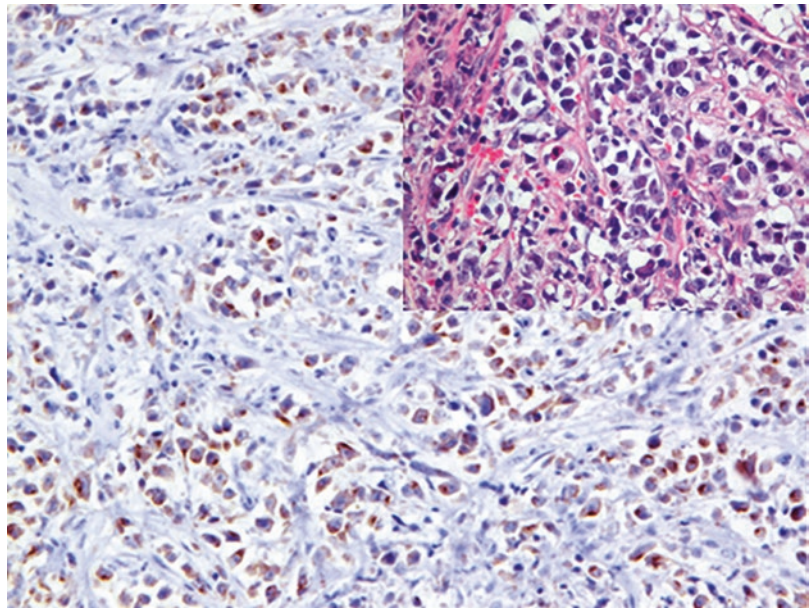


Fig. 16.14 Anaplastic large cell lymphoma with ALK -positive lymphoma cells

Diagnostic Approach Anaplastic lymphoma kinase (ALK) clustered as CD246 is a tyrosine kinase receptor expressed during the embryogenesis and remains positive in glial cells of CNS. ALK is negative in normal lymphoid tissue but expressed in some lymphoma types, namely, anaplastic large cell lymphoma, due to the activation of the ALK transcription caused by a potent promotor as a result of the t(2;5) translocation or another equivalent translocation [43]. ALK is also positive in the inflammatory myofibroblastic tumor also associated with the same translocation [44].

A strong ALK expression is also characteristic for the ALK-positive large B-cell lymphoma. This rare lymphoma type lacks the t(2;5) translocation and is consistently CD30 negative (Fig. 16.14).

T-Cell Leukemia Protein 1 (TCL-1): TCL-1 is an oncoprotein normally expressed in the early embryogenesis of lymphocytes. TCL-1 is overexpressed in the cells of T-cell prolymphocytic leukemia as a result of the t(14;14)(q11;q32) rearrangement specific for this leukemia type. Other T-cell lymphoma types usually lack the TCL-1 positivity. TCL-1 is expressed in different lymphoma types of B-cell origin including follicular lymphoma, Burkitt lymphoma, mantel cell lymphoma, CLL, hairy cell leukemia, and diffuse large cell lymphoma, whereas marginal zone lymphoma, CD30+ anaplastic lymphoma, and plasma cell tumors are constantly negative for TCL-1.

The expression of TCL-1 is also characteristic for testicular intratubular germ cell neoplasms and seminoma.

16.5 Markers and Immunoprofile of NK-Cell Neoplasms

Immunohistochemical Markers for NK-Cell Lymphoma CD2, CD3, CD56, cytotoxic molecules (TIA-1, granzyme B, perforin), and EMA [8, 10].

CD56 (N-CAM; NKH1)		
Expression pattern: membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
NK lymphomas, multiple myeloma, acute and chronic myeloid leukemia, neuro-endocrine tumors (small cell carcinoma, carcinoid and Merkel cell carcinoma), pheochromo-cytoma, neuroblastoma, ovarian sex cord-stromal tumors	Synovial sarcoma, embryonal and alveolar rhabdomyo-sarcoma, angiosarcoma, solitary fibrous tumor, chordoma, epithelioid sarcoma, Ewing's sarcoma/PNET, medulloblastoma, schwannoma and neurogenic sarcoma, astrocytomas, ependymoma, meningioma, retinoblastoma, paraganglioma, melanoma, mesothelioma, bile duct adenoma	NK cells, activated T cells, cerebellum and brain cortex, neuro-muscular junctions, neurons, intestinal ganglion cells, neuroendo-crine tissue, thyroid follicular, epithelium, hepatocytes, epithelium of renal tubules, osteoblasts
Positive control: brain tissue/intestinal ganglion cells		

Diagnostic Approach CD56 (neural cell adhesion molecule, N-CAM) is a transmembrane adhesion molecule and a member of the Ig superfamily involved in the development of neural cells and differentiation of neural tissue. Normally, CD56 is expressed on the membrane of neuroectodermal cells, NK cells, activated T cells, myoblasts, and skeletal muscle. CD56 is an important marker for NK-cell lymphoma and also a very helpful marker for the diagnosis of pulmonary and extrapulmonary small cell carcinomas. CD56 is also a sensitive but less specific marker for ovarian sex cord-stromal tumors (see related section).

Diagnostic Pitfalls CD56 is an unspecific marker with a very wide expression spectrum. It is found in a small subset of CD4- and CD8-positive T cells and plasma cells. CD56 is also expressed on the cells of multiple myeloma, whereas CD56-negative myeloma is found to have a poor prognosis. CD56 may be also expressed on other tumors with similar morphology such as embryonal rhabdomyosarcoma, neuroblastoma, malignant melanoma neurogenic sarcoma, and synovial sarcoma which to consider in the differential diagnosis [36, 45].

Cytotoxic Molecules (Granzyme B, Perforin, and TIA-1) Antibodies to the cytotoxic molecules are important markers for the diagnosis of T cell and NK lymphomas. Perforin granzyme B and TIA-1 are the most popular cytotoxic molecules used in routine immunohistochemistry.

Perforin: Perforin is a cytolytic pore-forming protein found in the granules of cytotoxic T-lymphocytes. It is able to perforate a pore in the membrane of targeted cells.

Granzyme B: Granzyme B is a serine protease stored in specialized lytic granules of cytotoxic T-lymphocytes and natural killer cells together with perforin. Granzyme B seems to enter the target cell through a perforin-caused transmembrane pore to induce DNA fragmentation initiating apoptosis of targeted cells.

TIA-1: TIA-1 (also known as nucleolysin) is a cytotoxic granule-associated protein expressed in natural killer cells and cytotoxic T-lymphocytes. TIA-1 has a nucleolytic activity against targeted cells initiating apoptosis. TIA-1 is also used to label tumor-infiltrating lymphocytes.

Immunoprofile of T-cell and NK-cell neoplasms				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
Precursor T-cell lymphoblastic leukemia/lymphoma	*TdT*, CD7, *CD2* Proliferation index (Ki-67): 40–80%	CD3 (cytoplasmic), CD1a, *CD10*, CD4, CD5, CD8, CD33, CD34, CD99, Fli-1, LMO2	CD13, CD15	PAX-5, CD19, MPO
T-cell prolymphocytic leukemia	CD2, CD5, CD7, CD43, *TCL-1*	CD3, CD4	CD8	CD1a, CD10, CD25, CD28, CD56, TdT
T-cell large granular lymphocytic leukemia	CD2, *CD3*, CD5, CD8 (in the common type), CD16, cytotoxic molecules (TIA-1, perforin, granzyme B)	CD5, CD4, CD57 (in the common and NK-cell types)	CD56, CD56 (+ in NK-cell type), CD4 (+ in rare types)	CD7, CD10, CD25
Adult T-cell lymphoma (HTLV1+)	CD2, *CD3*, *CD4*, CD5, CD25			CD7, CD8
Extranodal NK-/T-cell lymphoma, nasal type	*CD2*, *CD3ε*, CD43, *CD56*, *cytotoxic molecules* (TIA-1, perforin, granzyme B), EBV	CD7		*CD3*, CD4, CD5, CD8, TdT
Peripheral T-cell lymphoma (NOS)	CD2, *CD3*, CD4, CD5	CD7	CD25, CD30, CD134	*ALK*, CD8, CD15[a], CD19, CD20[b]
Angioimmunoblastic T-cell lymphoma	CD2, *CD3*, CD4, CD5, CD7, *CD10*, CD28, *PD-1 (CD279)*, *bcl-6*, *CXCL13*[c] Expanded CD21- and CD23-positive meshwork of follicular dendritic cells		CD8, CD10, CD30 EBV+ B-cell blasts	CD15
Follicular T-cell lymphoma	CD3, CD4, *CD10*, *PD1 (CD279)*, *bcl-6*, *CXCL13*[c]			
Nodal peripheral T-cell lymphoma TFH phenotype	CD3, CD4, *CD10*, *bcl-6*, *PD-1 (CD279)*, *CXCL13*[c]			
Mycosis fungoides/ Sézary syndrome	CD2, *CD3*, CD5, CD4, CD45RO Proliferation index (Ki-67): <5%		CD7	CD8, CD25
Enteropathy-associated T-cell lymphoma	CD2, *CD3*, CD7, CD103	CD30, cytotoxic molecules (TIA-1, perforin, granzyme B)	CD8	CD4, CD5, CD56
Monomorphic epitheliotropic intestinal T-cell lymphoma	CD2, CD3, *CD8*, *CD56*			CD4
Indolent T-cell lymphoproliferative disorder of the GI tract	CD2, CD3, CD8	CD5, CD7, TIA-1		CD4, CD30, CD56
Hepatosplenic γδ T-cell lymphoma	CD2, *CD3*, CD43, CD45RO, TIA-1	CD7, CD56	CD8, CD16, CD5, CD11c, CD11b	CD4, CD5, perforin, granzyme
Anaplastic large cell lymphoma, ALK positive	*ALK*, *CD30*, clusterin[d], CD43, *cytotoxic molecules* (TIA-1, perforin, granzyme B)	CD2, CD4, CD25, CD45, EMA, galectin-3	CD3, CD5, CD7, CD15, fascin, bcl-6	CD8, CD20, CD28, PAX-5

Immunoprofile of T-cell and NK-cell neoplasms				
Anaplastic large cell lymphoma, ALK negative	*CD30*, clusterin[d], CD43, *cytotoxic molecules* (TIA-1, perforin, granzyme B)	CD2, CD4, CD25, CD45, EMA, galectin-3	CD3, CD5, CD7, CD15, fascin, bcl-6	*ALK*, CD8, CD20, CD28, PAX-5
Primary cutaneous anaplastic CD30-positive T-cell lymphoproliferative disorders – Lymphomatoid papulosis – Primary cutaneous anaplastic large cell lymphoma	*CD30*, CD4	CD45, CD25, cytotoxic molecules (TIA-1, perforin, granzyme B)	CD2, CD3, CD5, CD7	Clusterin, CD8, CD15, EMA, CD246 (ALK, p80), PAX-5
Subcutaneous (panniculitis-like) T-cell lymphoma	CD2, CD3, CD8, CD43, CD45, cytotoxic molecules (TIA-1, perforin, granzyme B)	CD5, CD7, CD25	CD30	CD4
Primary cutaneous gamma delta T-cell lymphoma	CD2, CD3, CD7, CD56, cytotoxic molecules (TIA-1, perforin, granzyme B)		CD8	CD4, CD5
Primary cutaneous CD8-positive aggressive epidermotropic cytotoxic T-cell lymphoma	CD3, CD8, cytotoxic molecules (TIA-1, perforin, granzyme B)	CD7	CD2	CD4, CD5
Primary cutaneous acral CD8-positive lymphoma	CD8	CD3, CD5, CD7, cytotoxic molecules (TIA-1, perforin, granzyme B)	CD4	CD30, CD56, EBV
Primary cutaneous CD4-positive small/medium T-cell lymphoproliferative disorder	CD3, CD4			CD8, CD30
Hydroa vacciniforme-like lymphoproliferative disorder	CD8, EBV	Cytotoxic molecules (TIA-1, perforin, granzyme B)		
Lymphomatoid papulosis	CD4, CD30[e]	CD2, CD3		CD8, ALK
Aggressive NK-cell leukemia	CD2, CD3ε, CD16, CD30 (only in large transformed cells), CD56 cytotoxic molecules (TIA-1, granzyme B)	CD8, EMA	CD7, CD16	CD3, CD4, CD5, CD8, CD57
Breast implant-associated anaplastic large cell lymphoma	CD2, CD4, CD5, CD30			CD10, ALK

[a]CD15 may be expressed in large cells of peripheral T-cell lymphoma
[b]B-cell antigens may be expressed in very rare cases (<5%) of peripheral T-cell lymphoma
[c]Chemokine (C-X-C motif) ligand 13 [46]
[d]Golgi staining pattern
[e]CD30 positive only in RS-like cells of type A lesion

16.6 Markers and Immunoprofile of Hodgkin's Lymphoma

16.6.1 Diagnostic Antibody Panel for Classical Hodgkin's Lymphoma

CD15, CD30, MUM-1, IMP3, fascin, and J-chain [47–49].

16.6.2 Diagnostic Antibody Panel for Nodular Lymphocyte-Predominant Hodgkin's Lymphoma

CD19, CD20, PAX-5, J-chain, BOB.1, Oct-2, and EMA [47].

CD15		
Expression pattern: membranous/cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Hodgkin's lymphoma (Reed-Sternberg cells), myeloid leukemia	Adenocarcinoma, sweat and sebaceous gland tumors, thymoma, ovarian carcinoma, renal cell carcinoma, thyroid carcinoma, peripheral T-cell lymphoma, ALCL	Granulocytes and precursors (neutrophils and eosinophils), monocytes, activated B and T cells, proximal tubules of kidney, intestinal Paneth cells
Positive control: appendix		

Diagnostic Approach CD15 (X hapten) is a cell surface glycoprotein involved in the regulation of neutrophil functions. CD15 is frequently used as a marker for normal and neoplastic myeloid cells and monocytes. In combination with CD30, CD15 is commonly used as a marker for Reed-Sternberg cells in classical Hodgkin's lymphoma (Fig. 16.15). CD15 is also expressed on different carcinoma types but constantly negative in mesothelioma. Carcinomas positive for CD15 reported to have worse prognosis.

Diagnostic Pitfalls In view of the fact that CD15 is expressed in different hematopoietic

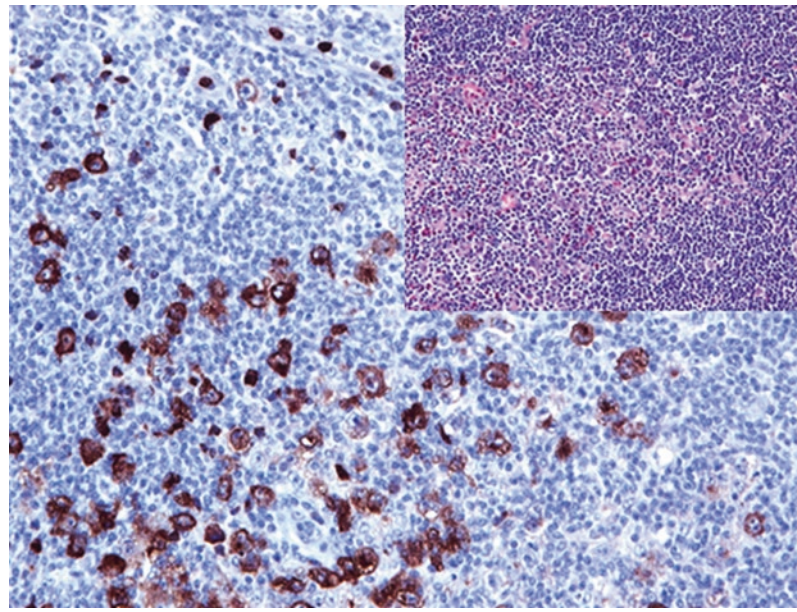


Fig. 16.15 CD15-positive Hodgkin cells in classical Hodgkin's lymphoma

and non-hematopoietic neoplasms including adenocarcinomas, it is important to keep in mind possible differential diagnoses and to support the final diagnosis by other more specific antibodies.

CD30		
Expression pattern: membranous/cytoplasmic paranuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Anaplastic large cell lymphoma, Reed-Sternberg cells in classic Hodgkin's lymphoma, primary mediastinal large B-cell lymphoma	Embryonal carcinoma, systemic mastocytosis, NK-/T-cell lymphoma, nasopharyngeal carcinoma, pancreatic adenocarcinoma melanoma, angiosarcoma, mesothelioma	Granulocytes, monocytes, activated B, T, and NK cells, small subset of plasma cells, exocrine pancreas glands, Purkinje cells of the cerebellum, cortical neurons, decidual cells
Positive control: embryonal carcinoma		

Diagnostic Approach CD30 (Ki-1)—also known as lymphocyte activation antigen—is a transmembrane glycoprotein receptor and member of the tumor necrosis factor superfamily participating in the regulation of cell transformation, antibody response, and apoptosis. CD30 is normally expressed in activated B, T, and NK cells. One of the major utilities of CD30 in routine immunohistochemistry is to highlight Hodgkin cells and multinucleated Reed-Sternberg cells in different types of classical Hodgkin's lymphoma (Fig. 16.16). CD30 is also a diagnostic marker for anaplastic large cell lymphoma and primary mediastinal large B-cell lymphoma as well as high malignant types of systemic mastocytosis [50].

The expression of CD30 is not restricted to lymphoid tissue and lymphoid neoplasms but also found in other different epithelial and mesenchymal tumors [51]. CD30 is a useful marker for the diagnosis of embryonal carcinoma. CD30 labels other carcinoma types such as nasopharyngeal carcinoma and pancreatic adenocarcinoma. In mesenchymal tumors, CD30 labels about 30% of angiosarcoma.

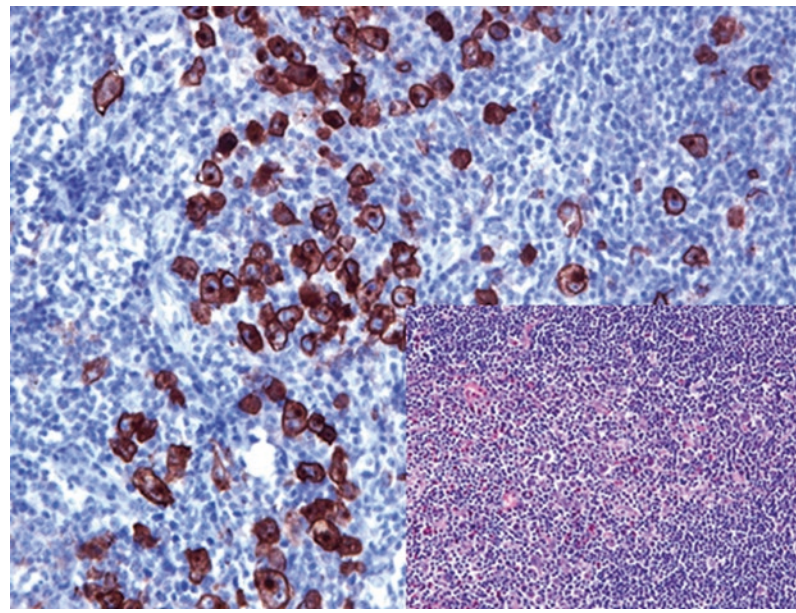


Fig. 16.16 CD30 expression in Hodgkin cells of classical Hodgkin's lymphoma

Diagnostic Pitfalls CD30-positive cells may be found in different T- and B-lymphoma types. CD30 stains also activated T and B cells in reactive lymph nodes, spleen, thymus, and tonsil; consequently, not all CD30-positive cells are Hodgkin cells.

Fascin (actin-bundling protein; p55)		
Expression pattern: membranous/cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Reed-Sternberg cells in classic Hodgkin's lymphoma, anaplastic large cell lymphoma, follicular and interdigitating dendritic cell tumors	Adenocarcinomas of the breast, colon, biliary tract, pancreas, lung, ovary, and skin; papillary transitional cell carcinoma of the bladder; diffuse large B-cell lymphoma; synovial sarcoma	Interdigitating and follicular dendritic cells, endothelial cells, EBV infected B-lymphocytes
Positive control: lymph node		

Diagnostic Approach Fascin is an actin-binding protein involved in cell adhesion and motility. It is normally expressed in interdigitating and follicular dendritic cells and variably in endothelial cells but constantly negative in lymphocytes, plasma cells, and myeloid cells. Fascin is a good marker for Reed-Sternberg cells in classical Hodgkin's lymphoma. It is also expressed on the membrane of anaplastic large cell lymphoma and subtypes of diffuse large B-cell lymphoma.

Fascin is constantly negative in normal epithelium but positive in many types of transformed or neoplastic epithelium [52]. This phenomenon may be used for the differentiation between hyperplastic and neoplastic urothelium.

Diagnostic Pitfalls Because of the wide expression spectrum of fascin, many differential diagnoses must be considered in the interpretation of the fascin immunostain. In addition to Reed-Sternberg cells, fascin-positive cells in lymph nodes maybe activated B-lymphocytes, cells of diffuse large B-cell lymphoma, or even disseminated cells of metastatic adenocarcinoma.

Insulin-Like Growth Factor II mRNA-Binding Protein 3 (IMP3): IMP3 is a cytoplasmic protein mediating RNA trafficking and cell growth, highly expressed in the early embryogenesis. Benign adult tissue usually lacks the expression of IMP3 with the exception of fibroblasts, subset of lymphocytes (mainly germinal center lymphocytes), ovarian and testicular tissue, placenta, and brain. IMP3 is expressed in different premalignant and malignant lesions. IMP3 is positive in different carcinoma types including pulmonary carcinoma, esophageal and pancreatic carcinoma, cervical and endometrial carcinoma, transitional cell carcinoma, renal cell carcinoma, and neuroendocrine carcinoma.

In routine immunohistochemistry, IMP3 is used to discriminate between malignant and reactive proliferative lesions. It is a useful marker to discriminate between pancreatic adenocarcinoma positive for IMP3 and inflammatory pancreas lesions usually negative for IMP3. IMP3 selectively stains Hodgkin and Reed-Sternberg cells in both classical Hodgkin's lymphoma and nodular lymphocyte-predominant Hodgkin's lymphoma (Figs. 16.17 and 16.18).

Diagnostic Pitfalls IMP3 may be positive in other extrafollicular blasts and must be used with other more specific markers to label Hodgkin cells.

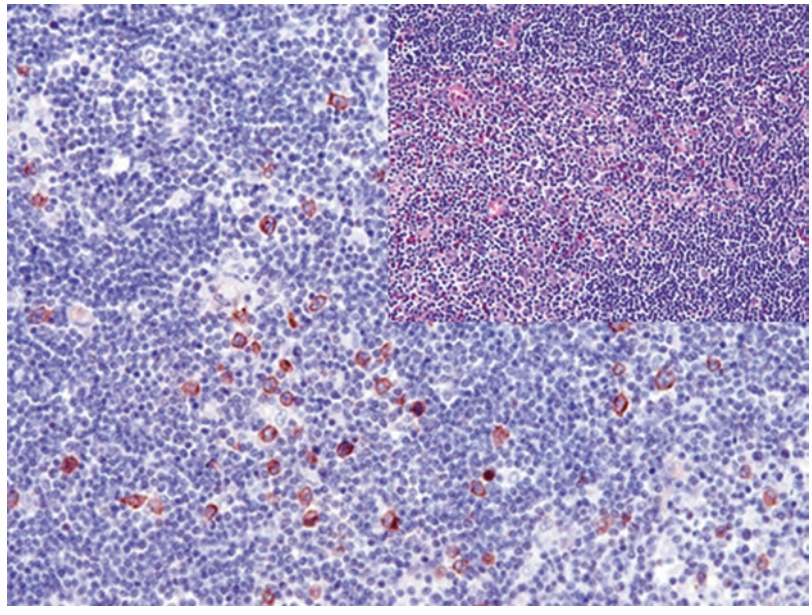


Fig. 16.17 IMP3 selectively labels in Hodgkin cells in classical Hodgkin's lymphoma

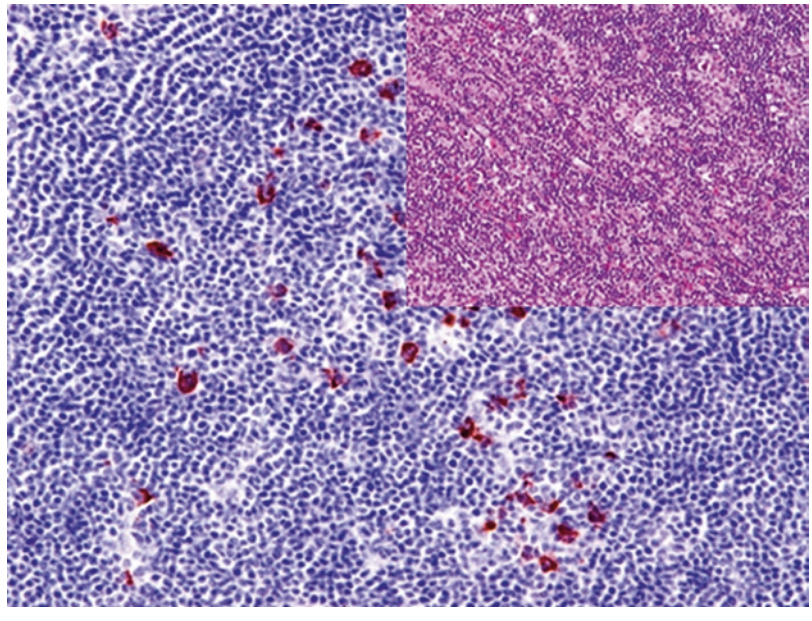


Fig. 16.18 IMP3 selectively labels in Hodgkin cells in nodular lymphocyte-predominant Hodgkin's lymphoma

Immunoprofile of Hodgkin's lymphoma				
Tumor type	+ in >90% (+)	+ in 50–90% (+/−)	+ in 10–50% (−/+)	+ in <10% (−)
Classical Hodgkin's lymphoma (Hodgkin and Reed-Sternberg cells[a]) in classical subtypes – Nodular sclerosis – Lymphocyte rich classic – Mixed cellularity – Lymphocyte depleted – Unclassifiable	*CD30*, IMP3, fascin	*CD15*, CD83, *PAX-5*, *MUM-1*, CD138, CD200, HLA-DR, EBV (LMP1)	CD20, CD79	CD45, Oct-2, BOB.1, J-chain, PU.1, EMA, bcl-6, CD22, ALK
Nodular lymphocyte-predominant Hodgkin's lymphoma (lymphocytic/histiocytic cells[a]) or popcorn cells (L&H cells)	CD19, CD20, CD22, CD45, CD86, PU.1, *Oct-2*, PAX-5, BOB.1, *J-chain*, IMP3	CD75, CD79a, CD40, bcl-6, *EMA*		CD10, CD15, fascin, MUM-1, CD30, CD138, CD200, ALK (p80), EBV

[a]Usually negative IgH and TCR gene rearrangements

References

1. Higgins RA, Blankenship E, Kinney MC. Application of immunohistochemistry in the diagnosis of non-Hodgkin and Hodgkin lymphoma. Arch Pathol Lab Med. 2008;132:441–61.
2. Zhao XF. Pitfalls in diagnostic hematopathology: part I. Int J Clin Exp Pathol. 2009;2:11–20.
3. Zhao XF. Pitfalls in diagnostic hematopathology: part II. Int J Clin Exp Pathol. 2010;3:39–46.
4. Buresh CJ, Oliai BR, Miller RT. Reactivity with TdT in Merkel cell carcinoma. A potential diagnostic pitfall. Am J Clin Pathol. 2008;129:894–8.
5. Sur M, AlArdati H, Ross C, et al. TdT expression in Merkel cell carcinoma: potential diagnostic pitfall with blastic hematological malignancies and expanded immunohistochemical analysis. Mod Pathol. 2007;20:1113–20.
6. Ordi J, Romagosa C, Tavassoli FA, et al. CD10 expression in epithelial tissues and tumors of the gynecologic tract. A useful marker in the diagnosis of mesonephric, trophoblastic, and clear cell tumors. Am J Surg Pathol. 2003;2:178–86.
7. Borscheri N, Roessner A, Röcken C. Canalicular immunostaining of neprilysin (CD10) as a diagnostic marker for hepatocellular carcinomas. Am J Surg Pathol. 2001;25:1297–303.
8. Higgins RA, Blankenship JE, Kinney MC. Application of immunohistochemistry in the diagnosis of non-Hodgkin and Hodgkin lymphoma. Arch Pathol Lab Med. 2008;132:441–61.
9. Chu PG, Arber DA, Weiss LM. Expression of T/NK-cell and plasma cell antigens in non-hematopoietic epithelioid neoplasms. An immunohistochemical study of 447 cases. Am J Clin Pathol. 2003;120:64–70.
10. Sweedlow SH, Campo E, Pileri SA, et al. The 2016 revision of the world health organization classification of lymphoid neoplasms. Blood. 2016;127:2375–90.
11. Masir N, Marafioti T, Jones M, et al. Loss of CD19 expression in B-cell neoplasms. Histopathology. 2006;48:239–46.
12. Jensen KC, Higgins JPT, Montgomery K, et al. The utility of PAX5 immunohistochemistry in the diagnosis of undifferentiated malignant neoplasms. Mod Pathol. 2007;20:871–7.
13. Feldman AL, Dogan A. Diagnostic uses of Pax5 immunohistochemistry. Adv Anat Pathol. 2007;14:323–34.
14. Kolhe R, Reid MD, Lee JD, et al. Immunohistochemical expression of PAX5 and TdT by Merkel cell carcinoma and pulmonary small cell carcinoma: a potential diagnostic pitfall but useful discriminatory marker. Int J Clin Exp Pathol. 2013;6(2):142–7.
15. Sullivan LM, Atkins KA, LeGallo RD. PAX immunoreactivity identifies alveolar rhabdomyosarcoma. Am J Surg Pathol. 2009;33:775–80.
16. Morgenstern DA, Gibson S, Sebire NJ, Anderson J. PAX5 expression in rhabdomyosarcoma. Am J Surg Pathol. 2009;33:1575–7.
17. Matutes E. New additions to antibody panels in the characterization of chronic lymphoproliferative disorders. J Clin Pathol. 2002;55:180–3.
18. Gladkikh A, Potashnikova D, Korneva E, et al. Cyclin D1 expression in B-cell lymphomas. Exp Hematol. 2010;38(11):1047–57.
19. Mozos A, Royo C, Hartmann E, et al. SOX11 expression is highly specific for mantle cell lymphoma and identifies the cyclin D1 negative subtype. Haematologica. 2009;94(11):1555–62.
20. Chen Y-H, Gao J, Fan G, et al. Nuclear expression of Sox11 is highly associated with mantle cell lymphoma but is independent of t(11,14)(q13;q32) in non-mantle cell B-cell neoplasms. Mod Pathol. 2010;23:105–12.
21. Soldini D, Valera A, Sole C, et al. Assessment of SOX11 expression in routine lymphoma tissue sections. Characterization of new monoclonal antibodies for diagnosis of mantle cell lymphoma. Am J Surg Pathol. 2014;38:86–93.

22. Ohno H. Pathogenetic role of BCL-6 translocation in B-cell non-Hodgkin's lymphoma. Histol Histopathol. 2004;19:637–50.
23. Went PT, Zimpfer A, Pehrs AC, et al. High specificity of combined TRAP and DBA.44 expression for hairy cell leukemia. Am J Surg Pathol. 2005;29(4):474–8.
24. van den Brand M, van Krieken J. Recognizing nodal marginal zone lymphoma: recent advances and pitfalls. A systemic review. Haematologica. 2013;98(7):1003–13.
25. Faline B, Agostinelli C, Bigerna B, et al. IRTA1 is selectively expressed in nodal and exranodal marginal zone lymphomas. Histopathology. 2012;61(5): 930–41.
26. Younes SF, Beck AH, Ohgami RS, et al. The efficacy of HGAL and LMO2 in the separation of lymphomas derived from small B cells in nodal and extranodal sites, including bone marrow. Am J Clin Pathol. 2011;135:697–708.
27. Natkunam Y, Sh Z, Mason DY, et al. The oncoprotein LMO2 is expressed in normal germinal-center B cells and in human B-cell lymphomas. Blood. 2007;109(4):1636–42.
28. Gratzinger D, Sh Z, West R, et al. The transcription factor LMO2 is a robust marker of vascular endothelium and vascular neoplasms and selected other entities. Am J Clin Pathol. 2009;131:264–78.
29. Natkunam Y, Lossos IS, Taidi B, et al. Expression of the human germinal center-associated lymphoma (HGAL) protein, a new marker of germinal center B-cell derivation. Blood. 2005;105(10): 3979–86.
30. Goteri G, Lucarine G, Zizzi A, et al. Comparison of germinal center markers CD10, BCL6 and human germinal center-associated lymphoma (HGAL) in follicular lymphoma. Diagn Pathol. 2011;6:97.
31. Tandon B, Peterson L, Gao J, et al. Nuclear overexpression of lymphoid-enhancer-binding factor 1 identifies chronic lymphocytic leukemia/small lymphocytic lymphoma in small B-cell lymphomas. Mod Pathol. 2011;24(11):1433–43.
32. Kermanshahi TR, Jayachandran P, Chang DT, Pai R. LEF-1 is frequently expressed in colorectal carcinoma and not in other gastrointestinal tract adenocarcinomas: an immunohistochemical survey of 602 gastrointestinal tract neoplasms. Appl Immunohistochem Mol Morphol. 2014;22(10):728–34.
33. Meyer PN, Fu K, Greiner TC, et al. Immunohistochemical methods for predicting cell of origin and survival in patients with diffuse large B-cell lymphoma treated with rituximab. J Clin Oncol. 2010;29:200–2007.
34. Matutes E. New additions to antibody panels in the characterization of chronic lymphoproliferative disorders. J Clin Pathol. 2002;55:180–3.
35. Torlakovic E, Slipicevic A, Florenes V, et al. Fli-1 expression in malignant melanoma. Histol Histopathol. 2008;23:1309–14.
36. Chu PG, Arber DA, Weiss LM. Expression of T/NK-cell and plasma cell antigens in non-hematopoietic epithelioid neoplasms. An immunohistochemical study of 447 cases. Am J Clin Pathol. 2003;120: 64–70.
37. Nunez AL, Siegal GP, Reddy VVB, et al. CD138 (syndecan-1) expression in bone-forming tumors. Am J Clin Pathol. 2012;137:423–8.
38. Natkunam Y, Warnke RA, Montgomery K, et al. Analysis of MUM1/IRF4 protein expression using tissue microarrays and immunohistochemistry. Mod Pathol. 2001;14:686–94.
39. Ning S. IRF4 as an oncogenic biomarker for hematological malignancies. J Oncobiomarkers. 2013;1(1): 1–6.
40. Shanks JH, Baneriee SS. VS38 immunostaining in melanocytic lesions. J Clin Pathol. 1996;49:205–7.
41. Pileri SA. Follicular helper T-cell related lymphomas. Blood. 2015;126(15):1733–4.
42. Islam A, Vladutiu AO, Donahue T, et al. CD8 expression on B cells in chronic lymphocytic leukemia. A case report and review of the literature. Arch Pathol Lab Med. 2000;124:1361–3.
43. Tuffaha M. Phenotypic and genotypic diagnosis of malignancies. Immunohistochemical and molecular approach in tumor diagnosis and detection of minimal residual cancer disease. Weinheim, Berlin: Wiley-VCH-Verlag; 2008.
44. Heim-Hall J, Yohe L. Application of immunohistochemistry to soft tissue neoplasms. Arch Pathol Lab Med. 2008;132:476–89.
45. Kontogianni K, Nicholson AG, Butcher D, Sheppard MN. CD56: a useful tool for the diagnosis of small cell lung carcinomas on biopsies with extensive crush artifact. J Clin Pathol. 2005;58:978–80.
46. Dupuis J, Boye K, Martin N, et al. Expression of CXCL13 by neoplastic cells in angioimmunoblastic T- cell lymphoma (AITL): a new diagnostic marker providing evidence that AITL derives from follicular helper T cells. Am J Surg Pathol. 2006;30(4): 490–4.
47. Pileri SA, Ascani S, Leoncini L, et al. Hodgkin's lymphoma: the pathologist's viewpoint. J Clin Pathol. 2002;55:162–76.
48. Bayerl MG, Bentley G, Bellan MC, et al. Lacunar and Reed-Sternberg-like cells in follicular lymphomas are clonally related to the centrocytic and centroblastic cells as demonstrated by laser capture microdissection. Am J Clin Pathol. 2004;122:858–64.
49. Tang H, Wei Q, Ge J, et al. IMP3 as a supplemental diagnostic marker for Hodgkin lymphoma. Hum Pathol. 2013;44(10):2167–72.
50. Sotlar K, Cerney-Reiterer S, Petet-Dutter K, et al. Aberrant expression of CD30 in neoplastic mast cells in high-grade mastocytosis. Mod Pathol. 2011;24(4): 585–95.
51. Alimachandani M, Wang ZF, Miettinen M. CD30 expression in malignant vascular tumors and its diagnostic and clinical implications: a study of 146 cases. Appl Immunohistochem Mol Morphol. 2014;22(5): 358–62.
52. Tong GX, Yee H, Chiriboga L, et al. Fascin-1 expression in papillary and invasive urothelial carcinomas of the urinary bladder. Hum Pathol. 2005;36(7):741–6.