Invited Review

Diagnosis of malignant lymphoma – An overview

INTRODUCTION

Malignant lymphomas are a heterogeneous group of neoplasm where the tumor cells resemble mature lymphocytes, histiocytes or their precursors. Lymphomas are classified into a fairly defined Hodgkin lymphoma (HL) and a much diverse non-HL (NHL). The traditional way of diagnosis, classification and prognostic assessment, based on morphologic features (cell size, characteristics of nucleus and cytoplasm and the distribution as nodular or diffuse) alone, is limited and requires immunohistochemistry, cytogenetics and molecular studies for accurate diagnosis. The following discussion is an overview of approach to immunodiagnosis of lymphoma and some of the common immunohistochemical markers used.

Morphologically, lymphoma fits into the larger category of "round cell tumors." When the neoplastic cells are small and round cells, they are inseparable from a variety of tumors (e.g., sarcomas such as rhabdomyosarcoma, Ewing sarcoma and nonsarcomatous tumors such as small cell carcinoma, melanoma and neuroblastoma). Immunohistochemistry plays a major role in the diagnostic workflow of lymphoma. The recent past has witnessed the introduction of numerous new and novel markers for diagnosis and immunoprofiling of lymphoma. Choice of markers, their sequential use (algorithm) and interpretation of the results require skill and experience. Nevertheless, the diagnostic workflow or algorithm should follow the sequence to address the below-said goals.

1. Establishing the lymphocytic lineage of neoplastic cells
2. Delineating the neoplastic lymphoid cells from nonneoplastic cells
3. Classify and subclassify the lymphoma to appropriate tumor type.

ESTABLISHING THE LYMPHOCYTIC LINEAGE

A primary panel of antibodies comprising *leukocyte common antigen (LCA or CD45)*, *pan-cytokeratin* (e.g., *AE1/AE3*), *S100 protein* and *vimentin* is sufficient to sort out most of the large round cell tumors. For small-to-intermediate round cell tumors, in addition to the above-said markers, desmin, *FLI1* and *CD99* may help if the diagnostic consideration includes tumors such as rhabdomyosarcoma, Ewing sarcoma or poorly differentiated synovial sarcoma.

LCA or *CD45* contains a group of antibodies which recognizes almost all lymphoid cells and their precursors, targeting a family of protein tyrosine phosphatase isoforms on the lymphocyte membrane. It is available as *CD45* or its restricted subsets *CD45RA, CD45RB* and *CD45RO*. It is worthy to note that only *CD45, CD45RB* and their cocktail (*CD45/CD45RB*) are considered as pan-leukocytic markers.[1]

The points to consider with respect to primary panel of antibodies are:

- The expression of *LCA* is found in more than 95% of the NHLs. Some lymphoblastic lymphomas and anaplastic large cell lymphomas (ALCLs) may be *LCA* negative. Such negative tumors require the use of *CD30, CD43* or other markers for diagnosis
- Similarly, Reed–Sternberg cells and their variants found in classical HLs do not express *LCA*
- Although strong *pan-cytokeratin* positivity tilts the diagnosis toward carcinoma, rare cases of anaplastic or plasmablastic lymphomas may show true keratin positivity. However, the later produces characteristic dot-like paranuclear staining instead of diffuse cytoplasmic staining found elsewhere
- Care should be taken while using alternative epithelial markers such as epithelial membrane antigen (*EMA*). Most ALCLs, plasma cell neoplasms, nodular lymphocyte-predominant HL (LPHL) and few T-cell-rich B-cell lymphomas are *EMA* positive
- Rarely, histiosarcoma may show focal *S100 protein* positivity.

LYMPHOMA VERSUS LYMPHOID HYPERPLASIA

At times, differentiating follicular lymphoma from reactive follicular hyperplasia, especially when the site involves lymph node, is a challenge. Histologically, preservation of

nodal architecture and sinus pattern, variation in the size and shape of the follicles and well-demarcated germinal centers exhibiting macrophages with cell debris (because of active phagocytosis) may tilt the diagnosis toward reactive follicular hyperplasia than a follicular lymphoma.[2]

Markers useful in differentiating follicular lymphoid hyperplasia and follicular lymphoma include *CD20, bcl-2, CD3, CD10, CD43* and *CD5*.[1]

COMMON MARKERS OF LYMPHOMA

B-cell markers

CD19, Pax-5 and *TdT* are early B-cell differentiation markers expressed in precursor B-cells. Later, *CD20, Pax-5* and *CD79a* control further B-cell differentiation and, are also considered as markers of B-cell lineage.

The marker *CD20* is positive in most of the B-cell lymphomas except mature B-cells/plasma cell neoplasms such as multiple myeloma/solitary plasmacytoma and plasmablastic lymphoma. *CD20* is negative in all the T-cell lymphomas. Among HL, *CD20* is consistently positive in nodular LPHL, whereas only 25% of Reed–Sternberg cells of classical HL are *CD20* positive.[3,4]

Like *CD20*, *Pax-5* is also positive in all the precursor and mature NHLs. Most of the Reed–Sternberg cells of classical HL are also *Pax-5* positive. However, it is negative in multiple myeloma, solitary plasmacytoma and a fraction of diffuse large B-cell lymphoma (DLBCL).[5]

CD79a is positive in all B-cell neoplasms except the fact that only 50% of the plasma cell neoplasms are positive. In HL, lymphocyte and histiocyte cells (L and H cells) are positive in nodular LPHL, whereas only 25 % positivity is found in classical HL.[6]

Most of the B-cell lymphomas strongly express *Oct-2* and *BOB.1*. Both markers are highly useful in rare cases of *CD20*-negative DLBCL and thus considered good "B-cell lineage" markers. In HL, the tumor cells (L and H cells) of nodular LPHL strongly express *Oct-2* and *BOB.1* and, therefore, useful in distinguishing them from classical Hodgkin cells.[7]

Immunoglobulins (IgM, IgG, IgA, IgD, kappa and *lambda)* may be detected in reactive plasma cells, plasma cell neoplasm and B-cell neoplasm such as plasmacytoid B-cell lymphoma. Light chain (*kappa* and *lambda*) may also be useful in determining the clonality of the B-cell proliferation or plasma cells.[8]

Hodgkin lymphoma markers

Most of the classical HLs are *CD15* positive, whereas nodular LPHLs are negative. Few cases of B-cell lymphomas, T-cell lymphoma and ALCL also express *CD15*. The marker *CD30* is positive in Reed–Sternberg cells of all classical HLs and all cases of ALCL but rarely expressed in other NHLs. *CD57* is primarily an NK-cell marker which is expressed in some T-lymphoblastic lymphoma and NK-cell neoplasms. With regard to HL, an increased number of *CD57*-positive cells are seen encircling the L and H cells of nodular LPHL. *Epstein–Barr Virus–Latent Membrane Protein (EBV-LMP)* is positive in EBV-related neoplasms such as classical HL, infectious mononucleosis and AIDS-related NHLs, whereas they are negative in nodular LPHL.[1,9]

T/NK-cell markers

Differentiation of T-cells starts at bone marrow and continues in the thymus. *CD7* is the earliest T-cell lineage marker to be expressed, followed by *CD2, CD5* and *CD3* in the bone marrow. In the thymus, they are further primed to co-express *CD4* and *CD8,* and as they enter the circulation, either one of *CD4* or *CD8* is expressed. A set of T-cells (γδ T-cells) accounting <5% fails to express *CD4, CD8* and *CD5*. NK-cells express *CD2, CD7, CD8, CD56* and *CD57*.

Although the expression of *CD7* is sustained throughout the T-cell differentiation, they are lost in most of the lymphomas except precursor T-lymphoblastic lymphoma and most NK-cell neoplasm.[10]

Unlike other markers, *CD3* is a T-cell lineage marker, the expression pattern of which is membranous, cytoplasmic or both. Interpretation of pattern of expression is pivotal since precursor T-cell lymphomas express as cytoplasmic positivity, whereas peripheral T-cell lymphomas express as membranous positivity.[1]

CD2 is a pan T-cell lineage marker which is positive in most of the lymphomas of T-cell lineage. *CD5* is expressed in most small lymphocytic lymphoma, mantle cell lymphoma and few cases of follicular center cell lymphoma. *CD5* is negative in Burkitt lymphoma, marginal zone lymphoma and multiple myeloma.

CD4 is expressed in most peripheral T-cell lymphoma, mycosis fungoides and HTLV-1-associated adult T-cell lymphoma but not in NK-cell neoplasm. Co-expression or simultaneous loss of both *CD4* and *CD8* is diagnostic of T-cell lymphoma.[1]

OTHER MARKERS USEFUL IN THE DIAGNOSIS OF LYMPHOMA

Bcl-1, otherwise known as cyclin D1, is a marker often expressed in mantle cell lymphoma and 25% of plasma cell neoplasms. *Bcl-2*, an anti-apoptotic protein, is normally expressed in a variety of cells including lymphoid tissue, whereas it is negative in reactive germinal centers which makes it a useful candidate to differentiate reactive lymphoid hyperplasia from follicular lymphoma, the latter being intensely positive. *Bcl-6* positivity is noted in germinal center and postgerminal center B-cell neoplasms. Most T-cell neoplasms are negative except half the cases of ALCL. All nodular LPHLs and a considerable number of classical HLs are positive for *Bcl-6*.[11]

CD10 is found in Burkitt lymphoma, follicular lymphoma and B-cell lymphoblastic lymphoma. Considerable cases of ALCL, DLBCL and multiple myeloma are also positive for *CD10*.[8]

Multiple myeloma oncogene (*MUM1*) is normally expressed in terminal stages of differentiation of B-cells in the germinal center and plasma cells. *MUM1* is strongly expressed in lymphoplasmacytic lymphoma, small lymphocytic lymphoma, a subset of follicular lymphoma, marginal zone B-cell lymphoma, diffuse large B-cell lymphoma and multiple myeloma. Nonetheless, M*UM1* is negative in B-lymphoblastic lymphoma and Burkitt lymphoma. Hodgkin and Reed–Sternberg cells are also positive for *MUM1*.[12]

CD138 (Syndecan-1) is a marker expressed in various distinct stages of normal B-cell differentiation, especially Pre-B-cell and immunoglobulin producing plasma cells, but not in T-cells or any other lineage. *CD138* marker is extremely useful in the diagnosis of multiple myeloma (plasmacytoma), lymphoplasmacytic lymphoma and plasmablastic lymphoma with plasmacytoid features. It should be noted to establish the lymphoid lineage of the tumor earlier since *CD138* is also positive in many nonhematolymphoid tumors including melanoma.[13]

CD43 is a marker positive in all T-cells, NK-cells and the respective neoplasms. Contrarily, most B-cells are negative except for very immature B-cells and some activated B-cells. Therefore, *CD43* usage is restricted *to CD20*-positive low-grade lymphomas, in particular those which fail to express *LCA (CD45)*.[14,15]

Anaplastic lymphoma kinase (ALK) is a marker overexpressed in most of the ALCLs and a subset of B-cell neoplasms. Since it is not found in any normal cells outside CNS, ALK positivity in a lymphoid tissue may essentially represent malignancy. Nevertheless, few cases of inflammatory myofibroblastic tumors are also positive for ALK.[16]

Terminal deoxynucleotidyl transferase (TdT) is a sensitive marker for immature lymphocytes and, therefore, positive in lymphoblastic lymphoma of both T- and B-cell lineage. However, care should be exerted to use TdT once the lymphocytic lineage of the tumor cell is established since it is also expressed in other round cell tumors such as medulloblastoma, rhabdomyosarcoma and Ewing sarcoma.[1]

Granzyme B, perforin and *TIA-1* are expressed in cytotoxic T-cells and NK-cells, and are useful in the diagnosis of cytotoxic T-cell lymphomas and NK/T-cell lymphomas.[1]

MYELOID MARKER

Markers such as *CD117, lysozyme, myeloperoxidase, CD33* and *hemoglobin* A are used as myeloid markers with varying degrees of sensitivity and specificity and their role in oral and maxillofacial region is limited.

HISTIOCYTIC AND DENDRITIC CELL MARKERS

CD1a is a relatively specific marker for Langerhans cells and its precursors. Occasionally, a subset of lymphoblastic lymphomas may show positivity for *CD1a*. Similarly, the marker *langerin* is a sensitive and specific marker for Langerhans cells. Both the markers are helpful in the diagnosis of Langerhans cell histiocytosis.

CD21/CD35 clusters are useful markers to identify the follicular dendritic cells and their neoplasms. However, a subset of B-cells and Reed–Sternberg cells of HL are also positive.

CD163 is a marker for macrophages and histiocytes, and it is useful in the diagnosis of Langerhans cell histiocytosis, histiocytic sarcoma and sinus histiocytosis with massive lymphadenopathy.[17]

CD123, though expressed in a variety of normal hematolymphoid cells, is expressed in unusual diseases such as Kikuchi disease and acute leukemia.[18]

CD68 is a marker for all cells of monocyte/macrophage lineage and their neoplasm. Yet, Langerhans cell and dendritic cells fail to express *CD68*. On the downside, dot-like cytoplasmic positivity may be noticed in mast cell neoplasm and a subset of NHLs.[19,20]

S100 protein is a reliable marker for Langerhans cells and dendritic cells and, therefore, useful for the diagnosis of Langerhans cell histiocytosis, sinus histiocytosis and dendritic cell sarcoma (focal staining in the later).

MAST CELL MARKERS

Tryptase is a specific marker of human mast cells, and it is useful in mast cell proliferative disorders such as malignant mastocytosis. However, mast cell neoplasms are extremely rare in the oral and maxillofacial region.[21]

CD117 (C-kit) is positive in some myeloid and erythroid precursor cells and mast cells and, therefore, helpful in the diagnosis of mast cell disorders.

CONCLUSION

Lymphoma is a complex pathology to diagnose, and available diagnostic markers are plenty. Understanding the differentiation of hematolymphoid cells and their immunoprofiling is of at most importance for the pathologist so as to form an appropriate diagnostic algorithm.

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