Malignancy of the Lymph Node: How General Practitioners and Pathologists can achieve a Definitive Diagnosis

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ABSTRACT

The lymph node plays an important role in the lymphatic spread of abnormal antigens from exogenous or endogenous sources, including infectious agents, foreign bodies, self-antigens, and malignant cells, by harboring various immune cells that react to abnormal antigens and their sources. This often leads to enlargement of the lymph node, also known as "lymphadenopathy." In this review article, malignancy of the lymph node is the main focus, especially regarding how general practitioners and pathologists can achieve a definitive diagnosis. The basic principle relies on the normal structure, cellular components, and functions of the lymph node as well as the types of malignancy found. Careful clinical history taking of any possible cause of lymphadenopathy warrants exclusion of any mimics of malignancy of the lymph node, including drug reactions and immunodeficiency states. An adequate cell or tissue sample allows pathologists to work efficiently by mastering the multimodality approach under good clinical collaboration. Effective communication between pathologists and physicians regarding relevant laboratory investigations should make it easier to diagnose a specific type of malignancy. This review article also focuses on how general pathologists handle cell or tissue samples by conventional morphologic evaluation and panels of immunohistochemistry so that general practitioners understand the diagnostic process and understand how to diagnose malignancy of the lymph node.

Keywords: Lymph node; malignancy; multimodality; morphology; immunohistochemistry; pathologic diagnosis
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INTRODUCTION

Malignancy of the lymph node can be divided into primary (the cellular components) and secondary or metastatic tumor, including leukemic infiltration. A primary malignancy of the lymph node is mostly the result of neoplastic lymphoid cell clone, best known as malignant lymphoma. Meanwhile, secondary malignancy is usually a metastatic tumor of nearby structures or at times an unknown primary site.[1] Leukemic infiltration of the lymph node is also a secondary malignancy, but terminology prefers to use infiltration (or involvement) to metastasis. However, this is uncommon prior to typical leukemic manifestation in general.[2] Leukemic infiltration, poorly-differentiated neuroendocrine carcinoma ("small cell carcinoma"), metastatic invasive lobular carcinoma, and other metastatic tumors may create difficulties in making a definitive diagnosis because they share morphologic similarities to malignant lymphomas. This particular group of tumor cells with small round cell morphology is often referred to as "small round-cell tumor (SRCT),[3,4] however, pathologists should pay close attention to the size of tumor cells because after provisional diagnosis,

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immunohistochemistry (immunostaining) can reveal diffuse large B-cell lymphoma (DLBCL). In such cases, discrepancy between provisional diagnosis of SRCT and final diagnosis of DLBCL can lead to a concern about the proficiency of the pathologist. In order to provide a definitive diagnosis of a malignancy in the lymph node, general practitioners and pathologists should understand the following topics.

Basic principles regarding the lymph node

The lymph node plays an important role in handling the lymphatic spread of abnormal antigens from exogenous or endogenous sources, including infectious agents, foreign bodies, self-antigens, and malignant cells, by harboring various types of immune cells that react to abnormal antigens. The process usually leads to enlargement of the lymph node, also known as "lymphadenopathy." Therefore, knowledge of the basic principles of normal structure, cellular components, and functions of the lymph node, as well as the types of malignancy commonly found in the lymph node should allow general practitioners and pathologists to make a definitive diagnosis.

I) Normal structure of the lymph node and types of malignancy found

Regarding primary or secondary malignancy of the lymph node, the normal structure of the lymph node can explain why tumor cells localized in lymph node sinuses tend to be metastatic tumors with lymphatic spreading. In addition, some lymphoma cells can have a lymph node sinus distribution mimicking metastatic tumor. When tumor cells are localized in the paracortex (interfollicular area or the area of the lymph node between the cortex and medulla), it can be suggestive of primary or secondary malignancy of the lymph node. Normally, the paracortex is predominantly occupied by T-cells (the "T-cell zone") but it is also the place where circulating cells enter the lymph node via postcapillary venules, commonly known as "high endothelial venules" (HEV) in immunology. Thus, not only T-cell lymphoma and B-cell lymphoma arising from a non-germinal center B-cell clone that are common in the paracortex, but also metastatic tumor cells with hematogenous spreading that can enter the lymph node via HEV in the paracortex. Certainly, leukemic cells can also reach the lymph node and cause lymphadenopathy via this route. The cortex, mostly occupied by B-cells and lymphoid follicles, is affected by malignant lymphomas, especially follicular lymphoma or other lymphomas arising from germinal center B-cells. Pathologists should be aware of the normal structure of the lymph node, especially the compartments commonly affected by various types of malignancy. The main challenge during microscopic examination of the lymph node is when tumor cells destroy normal structures of the lymph node (complete effacement of the lymph node) and the aforementioned clues cannot be used, leaving only morphologic evaluation of tumor cells as a possible solution.[1,5] (Table 1)

TABLE 1. Compartments of the lymph node and possible types of malignancy.

Compartment	Common malignancy	Less common malignancy
Lymph node sinus	Metastatic tumor with lymphatic spreading	Anaplastic large cell lymphoma, rare variant of diffuse large B-cell lymphoma, and Langerhans cell histiocytosis
Paracortex (interfollicular area)	T-cell lymphoma	B-cell lymphoma, metastatic tumor with hematogenous spreading, and leukemic infiltration
Cortex	Follicular lymphoma and others arising from germinal center B-cells	Nodular T-cell lymphoma (arising from T follicular helper cells) and follicular dendritic cell sarcoma
All compartments (diffuse effacement of the lymph node)	Any type of malignancy; definitive diagnosis depends on morphologic features and immunophenotypic findings of the tumor cells	

II) Cellular components of the lymph node and the types of malignancy commonly found

Cellular components of the lymph node are important for understanding why some types of malignancy occur more frequently. The lymph node cortex is predominantly a B-cell zone, while the paracortex is an T-cell zone. The medullary cords are predominantly occupied by plasma cells while the medullary and subcapsular lymph node sinuses are occupied by sinus histiocytes. Also, follicular dendritic cells mainly occupy the germinal centers of the lymphoid follicles. Less common in the lymph node are plasmacytoid dendritic cells and interdigitating reticulum cells. These cellular components of the lymph node work together to react to incoming antigens via lymphatics or HEV. The antigens arrive at the lymph node via lymphatics and trigger proliferation of sinus histiocytes so that the lymph node sinuses are dilated via accumulation of sinus histiocytes. At this point, without any detectable tumor cells, the enlarged lymph node caused by sinus histiocyte hyperplasia (or "sinus hyperplasia") is not diagnosed to have metastatic tumor.[1,5] Pathologists should avoid the terminology "sinus histiocytosis" because a general practitioner can get confused with "sinus histiocytosis with massive lymphadenopathy (SHML)" or "Rosai-Dorfman disease (RDD)." The author once found a patient who unfortunately received a course of local irradiation for an enlarged lymph node diagnosed as "sinus histiocytosis" because the radiotherapist misunderstood that it was "SHML" or "RDD." Fortunately, the patient was referred to Siriraj Hospital, the histologic slides were reviewed and a diagnosis of "sinus hyperplasia" of the lymph node was given instead of "sinus histiocytosis" to avoid misunderstanding as "SHML" or "RDD." Sometimes, lymphoma in a lymph node dissection specimen from patients who underwent tumor resection are overlooked as pathologists generally pay attention to metastatic tumors in lymph node sinuses.[6]

III) Functions of the lymph node and the types of malignancy found

The function of the lymph node in terms of immune reaction either helps or hampers diagnosis of malignancy. The most common immune reaction to tumor cells is involving tumor infiltrating lymphocytes (TIL), first described in malignant melanoma.[7] Therefore, assessment of TIL has been proposed in other types of malignancy as well.[8,9] In terms of morphologic evaluation of malignancy in the lymph node, TIL may lead to histologic features like lymphoepithelial carcinoma, indolent lymphoma, or T-cell rich variants in large cell lymphoma or classic Hodgkin lymphoma (CHL),[5] depending on the size, number, and morphology of tumor cells.

Epithelioid histiocytes (a pathology term) or activated macrophages (immunology term) sometimes intermingle with tumor and other immune cells in the lymph node. They can form tiny clusters, aggregates, sheets, or even granulomas, that lead to the wrong diagnosis of granulomatous lymphadenitis. In some places, where infectious diseases are common, coexisting tuberculosis and malignancy are found in the same lymph node.[10] When a lymph node with more clusters or aggregates of epithelioid histiocytes mixed with tumor cells, it can lead to the wrong diagnosis of granulomatous lymphadenitis. Therefore, pathologists should be aware of some tumors that have accompanying epithelioid histiocytes in clusters, aggregates, sheets, or even granuloma – germ cell tumor, CHL, and non-Hodgkin lymphoma (NHL) such as lymphoepithelioid lymphoma ("Lennert lymphoma"), a variant of peripheral T-cell lymphoma, angioimmunoblastic T-cell lymphoma, T-cell/histiocyte rich large B-cell lymphoma, Burkitt lymphoma, and small lymphocytic lymphoma/chronic lymphocytic leukemia (SLL/CLL).[5,11,12] Lastly, aggregates or sheets of epithelioid histiocytes can mimic metastatic carcinoma at times.

Tumor necrosis in the lymph node is occasionally seen with or without any preceding history of fine needle aspiration of the lymph node. At times, lymph node infarction can also occur. Careful evaluation for vascular occlusion can reveal tumor emboli or angiodestruction by tumor cells; the latter is more common in NHL.[5,13]

IV) Types of malignancy found in the lymph node

Malignant lymphomas and metastatic tumors are classified as primary or secondary malignancy, respectively. Malignant lymphomas at present are defined as a malignancy of lymphoid cells, which is different than in the past when it was a malignancy of the lymphoid tissue. Therefore, in the present classification of malignant lymphomas, we do not have histiocytic lymphoma, which was common in the 1960s according to the Rappaport classification for NHL. The different types of malignant lymphoma require clinical, morphologic, immunophenotypic, and genetic findings in order to make a definitive diagnosis according to the revised 4th edition of the WHO classification, published in 2017.[5] Malignant lymphoma can be divided into 3 types – B-cell lymphoma, T/NK cell lymphoma, and CHL – after excluding post-transplant lymphoproliferative disorder (LPD) and other iatrogenic immunodeficiency-associated LPD that can lead to varied pathologic findings, including any type of malignant lymphoma. A careful and complete clinical history review for immnosuppression is important in handling lymphadenopathy. Discontinuation of methotrexate or other immunosuppressive drugs can lead to improvement in clinical outcomes within one

week and spontaneous regression of the enlarged lymph node within three weeks.[5,14]

For general practitioners and pathologists, malignant lymphoma can be perplexing with 51 established entities plus six provisional entities of malignant lymphoma, according to the 2017 WHO classification.[5] A simplified version is presented in Table 2 with an emphasis on important issues that general practitioners and pathologists can help to manage efficiently for the lymphoma patients.

Regarding secondary malignancy of the lymph node, metastatic tumors are more common than leukemic infiltration or plasma cell myeloma (multiple myeloma).

TABLE 2. A simplified classification of malignant lymphomas for general practitioners and pathologists (modified from revised 4th edition of WHO classification, 2017)[5]

Clinical version	Morphologic version	Immunophenotypic version	Genetic version
Based on historical approach: Hodgkin vs Non-Hodgkin	Based on pattern: nodular vs diffuse	B-cell lymphoma - B-LBL - BL - DLBCL - FL - GZL - HGL - IVL - LPL - LYG - MCL - MZL - PBL - PEL - SLL	IGH gene, kappa Ig gene, lambda Ig gene BL: *MYC* DLBCL: *MYC; BCL2*; *BCL6; IRF4; MYD88*; FL: *BCL2* MCL: *CCND1* MZL: *BIRC3 (API2), MALT1, BCL10*
Based on clinical behavior: indolent, aggressive, or leukemic	Based on cell size: small vs large (including medium-sized).	T-cell lymphoma & NK/T-cell lymphoma - AITL - ALCL - ATLL - ENKT - HSTCL - ITL - MF/SS - PML - PTCL, NOS - SEBVT - SPTCL - T-LBL	TCR genes AITL: *RHOA, TET2, IDH2, DNMT3A, CTLA4* ALCL: *ALK, NPM1, BCL6, PTPN12, SERPINA1, CEBPB,* JAK/STAT pathway ATLL: *HBZ* HSTCL: *STAT5B*
Based on site of involvement: nodal vs extranodal	Based on nuclear features: blastic/blastoid vs mature	Hodgkin lymphoma - CHL - NLPHL	Clonal rearrangement of IGH gene by microdissection of LP & HRS cells
Based on identifiable causes: Breast implant-associated; chronic inflammation-associated; EBV-associated; *Helicobacter pylori*-associated; HHV-8-associated; HTLV-1-associated; MTX-associated; immunodeficiency-associated			

Abbreviations: AITL: Angioimmunoblastic T-cell lymphoma, ALCL: Anaplastic large cell lymphoma, ATLL: Adult T-cell leukemia/lymphoma, BL: Burkitt lymphoma, B-LBL: B-lymphoblastic lymphoma, CHL: Classic Hodgkin lymphoma, DLBCL: Diffuse large B-cell lymphoma, ENKT: Extranodal NK/T-cell lymphoma, nasal type, FL: Follicular lymphoma, GZL: Gray zone lymphoma (DLBCL vs CHL), HGL: High grade B-cell lymphoma (BL vs DLBCL), HRS: Hodgkin-Reed-Sternberg cells in CHL, HSTCL: Hepatosplenic T-cell lymphoma, IGH: Immunoglobulin heavy chain; ITL: Intestinal T-cell lymphoma, IVL: Intravascular large B-cell lymphoma, LP: LP cells in NLPHL, LPL: Lymphoplasmacytic lymphoma, LYG: Lymphomatoid granulomatosis, MCL: Mantle cell lymphoma, MF/SS: Mycosis fungoides/Sezary syndrome, MTX: Methotrexate; MZL: Marginal zone lymphoma, NLPHL: Nodular lymphocyte predominant Hodgkin lymphoma, PBL: Plasmablastic lymphoma, PEL: Primary effusion lymphoma, PML: Primary mediastinal large B-cell lymphoma, PTCL, NOS: Peripheral T-cell lymphoma, not otherwise specified, SEBVT: Systemic EBV+ T-cell lymphoma of childhood, SLL: Small lymphocytic lymphoma, SPTCL: Subcutaneous panniculitis-like T-cell lymphoma, TCR: T-cell receptor, T-LBL: T-lymphoblastic lymphoma

Cell type and origin (primary site) of metastatic tumors are important because they help guide specific treatment and management of patients. Without any clinical information, pathologists attempt to determine cell type of the metastatic tumor by morphology, histochemistry, immunostaining, and a genetic approach. Usually, it is not difficult to diagnose metastatic tumors in the lymph node because tumor cells are found primarily in lymph node sinuses. The tumor cells tend to form aggregates or sheets as they form tight junctions with nearby tumor cells. Moreover, the morphology of most metastatic tumor cells is different from that of immune cells in the lymph node. However, at times, the metastatic tumor cells can look like lymphoid cells or other accessory cells in the lymph node such as histiocytes, follicular dendritic cells, and interdigitating reticulum cells. Leukemic infiltration, poorly-differentiated neuroendocrine carcinoma ("small cell carcinoma"), metastatic invasive lobular carcinoma, and a number of other metastatic tumors cause difficulties in making a definitive diagnosis because they share morphologic similarities to malignant lymphoma. An experienced pathologist should spend time looking for clues in histologic sections such as immature eosinophils (eosinophilic myelocyte) in leukemic infiltration, nuclear debris along the blood vessel wall in small cell carcinoma, and a large PAS+ cytoplasmic globule by histochemistry in invasive lobular carcinoma.

In case of metastatic carcinoma to the lymph node, common types include adenocarcinoma, squamous cell carcinoma, neuroendocrine carcinoma, urothelial carcinoma, clear cell carcinoma, mucoepidermoid carcinoma, anaplastic carcinoma, and metastatic carcinoma from special types of salivary gland tumors, thyroid gland tumors, pancreatic cancers, gynecologic cancers, etc. In terms of other non-hematologic malignancy, metastatic melanoma, germ cell tumor, and sarcoma are quite common. At present, several types of sarcoma can be diagnosed, even with a core needle biopsy.[15] Sarcomas tend to spread via the hematogenous route, however, sarcomas with potential for lymph node metastasis include rhabdomyosarcoma, epithelioid sarcoma, clear cell sarcoma, synovial sarcoma, and vascular sarcoma.[16] Another important issue is how to determine the nature of the obtained tissue for pathologic examination. Sometimes, it is difficult to distinguish between a lymph node and a soft tissue mass, especially when the subcapsular lymph node sinus cannot be identified. A core needle biopsy is certainly more challenging than an incisional biopsy, especially when the tumor extends into the perinodal soft tissue. In practice, a schwannoma may look like an enlarged lymph node clinically, but surgeons can identify it as a soft tissue mass during excision. Anyway, a number of schwannoma can be missed and submitted to pathology laboratory as "a lymph node." Pathologists usually demonstrate that it is schwannoma – not a lymph node. A core needle biopsy of a schwannoma in most published articles is claimed to lead to more accurate diagnosis than fine needle aspiration due to adequate tissue for evaluation.[17]

Before the advent of immunostaining, histochemistry was used quite frequently. It helps in identifying mucin production in metastatic adenocarcinoma, melanin pigments in metastatic melanoma, or PAS+ intranuclear inclusion in neoplastic plasma cells of lymphoplasmacytic lymphoma, other small B-cell lymphoid neoplasm with plasmacytic differentiation, or even nodal involvement by plasma cell myeloma (multiple myeloma). However, in daily pathology practice at present, a panel of immunostaining is preferred and some pathologists have no experience to use histochemistry for identifying certain materials as mentioned above. Anyway, histochemistry is still worthy in places with limited resources.

A panel of immunostaining can be used in different morphologic settings to determine cell types in a malignancy of the lymph node. The principle is to apply a commercially available antibody specific to an antigen of interest in relation to tumor cells with the hope that, after a panel of antibodies, the immunophenotypic findings can be gathered and interpreted with morphologic correlations to achieve a definitive diagnosis of the type of malignancy. Tables 3 to 7 demonstrate panels of immunostaining proposed for use in various morphologic settings. If the clinical impression and morphology support each other, a pathologist can order a marker specific to the suspected tumor. For example, immunostaining for CD56 (neural cell adhesion molecule or NCAM) can be used in a suspected case of neuroblastoma. However, the expected negative marker, such as vimentin, should be included in the immunostaining panel because CD56 is not specific for neuroblastoma. The author once had a case of intra-abdominal mass in a child who was clinically suspected to have neuroblastoma. The marrow sample showed small blue cells that could be neuroblasts. Only immunostaining for CD56 was performed and tumor cells were positive for CD56 but the serum neuron specific enolase (NSE) level was not elevated that was unusual for metastatic neuroblastoma. So, exploratory laparotomy was performed to remove the tumor mass that was proven to be a sarcoma, probably embryonal rhabdomyosarcoma, supported by positive markers for muscle differentiation, vimentin, and CD56. It has been well documented that CD56 can be positive in a

TABLE 3. Panels of immunostaining proposed for use in undifferentiated neoplasm with large cell morphology (at least 3 times small lymphocyte in size) in the lymph node.[5,31]

Marker	Positive in	Remarks
First screening panel of immunostaining		
AE1/AE3	Epithelial tumors & epithelioid variant of sarcomas	Dot positive in neuroendocrine carcinoma (need to view at 40x magnification)
CD45 (leukocyte common antigen, LCA, or common leukocyte antigen, CLA)	Lymphoma & some leukemia	Can be negative in some lymphoma cells but when positive, a few positive tumor cells should be kept for hematologic malignancy
S-100	Melanoma, LCH, histiocytic sarcoma	Need both nuclear and cytoplasmic staining for positivity
Second screening panel of immunostaining (after failed first panel)		
ALK	ALK+ large B-cell lymphoma	
CD30	ALCL & lymphocyte-depleted CHL	
CD56	Large cell neuroendocrine carcinoma	
CD68	Histiocytic tumors & monoblastic sarcoma (leukemic infiltration by monoblasts)	
CD138	Plasmablastic lymphoma	
EMA	Epithelioid variant of sarcoma	
MPO	Myeloid sarcoma (leukemic infiltration by myeloblasts)	

Abbreviations: ALCL: Anaplastic large cell lymphoma, CHL: classic Hodgkin lymphoma, LCH: Langerhans cell histiocytosis

number of normal cells and several kinds of tumors, including embryonal rhabdomyosarcoma.[18] Thus, a good immunostaining panel should include not only a positive marker but also a negative one for tumors listed in the differential diagnosis.

Tumor markers do not have ideal specificity so that a complete investigation for primary sites is still needed. For example, NKX3.1 is believed to be a marker of prostatic origin in metastatic tumors, but only one out of 349 non-prostatic tumor tissue tested positive for NKX3.1 and that was a case of invasive lobular carcinoma of the breast.[19] Even CD45 (leukocyte common antigen), which is regarded as a highly specific marker in the hematolymphoid neoplasm, there are only seven definitive cases from five reports to date of CD45 expression on non-hematologic malignancy, including one primitive sarcoma (most probably rhabdomyosarcoma), four neuroendocrine carcinomas (including small cell carcinoma), one undifferentiated large cell carcinoma, and one NUT carcinoma; three cases were lymph node metastasis.[20-24] Panels of immunostaining as shown in Tables 3 and 4 provide both positive and negative results that should not have any conflicting immunophenotype. For example, AE1/AE3+ carcinoma cells should not have CD45 expression. When AE1/AE3+ CD45+ tumor cells are detected, a search for any technical error should be performed before acceptance of such an abnormal phenotype (AE1/AE3+ carcinoma with aberrant CD45 expression or CD45+ lymphoma with aberrant AE1/AE3 expression). Technical errors can be the cause of abnormal expression when immunostaining is performed manually, such as wrong slide labeling, applying wrong antibody in immunostaining, contamination of antibody by other antibodies during preparation of primary antibody for use, and interpretation of positive tissue control as the result of the test. All these technical errors can be resolved by using the fully automated immunostainer, except the last one that is caused by the pathologist who looks at positive tissue control placed on the same slide of the tested tissue. To prove that the tumor cells have aberrant expression, the pathologist reviews the histologic section and decides the type of malignancy. For example, if the

TABLE 4. A panel of immunostaining proposed for use in undifferentiated neoplasm with small cell morphology (1-2 times small lymphocyte in size) in the lymph node.[4,31]

Marker	Positive in	Remarks
First screening panel of immunostaining		
AE1/AE3	Epithelial tumor & epithelioid variant of sarcomas	Dot positive in small cell (oat cell) carcinoma (need to look at 40x magnification)
CD45 (leukocyte common antigen)	Lymphoma & some leukemia	Can be negative in some lymphoma cells but when positive, even a few positive tumor cells, should keep work-up for hematologic malignancy
S-100	Melanoma (small cell variant)	Need both nuclear and cytoplasmic staining for positivity
Second screening panel of immunostaining (after failed first panel)		
CD33	Myeloblastic infiltration	
CD34	Leukemic infiltration	
CD56	Neuroblastoma, embryonal rhabdomyosarcoma, BPDCN	
CD99	EWS/PNET	
CD123	BPDCN	
CD138	Nodal plasmacytoma	Nodal involvement by PCM
MPO	Myeloblastic infiltration	
TdT	Lymphoblastic leukemia/lymphoma	

Abbreviations: BPDCN: Blastic plasmacytoid dendritic cell neoplasm, EWS/PNET: Ewing sarcoma/Primitive neuroectodermal tumor, PCM: plasma cell myeloma (multiple myeloma)

morphology is that of carcinoma, then the tumor cells have aberrant CD45 expression. But if the morphology is that of malignant lymphoma, then the lymphoma cells have aberrant AE1/AE3 expression. If the morphology is not conclusive of any type of malignancy, then more markers are needed to support a diagnosis of malignancy. For example, if the AE1/AE3+ CD45+ tumor cells express CD20, CD10, CD79a, PAX5, MYC, BCL2, and BCL6, but are negative for EMA, CD3, CD5, and MUM1, then the diagnosis should be DLBCL with germinal center B-cell as the supposed cell of origin with triple protein expression of MYC, BCL2, and BCL6 proteins, and aberrant AE1/AE3 expression. But if the AE1/AE3+ CD45+ tumor cells express CK8/18, TTF-1, CK7, CD56, chromogranin, and synaptophysin but are negative for CK20, p40, p63, CD3, CD20, CD30, CD138, and MUM1, then the diagnosis should be metastatic carcinoma, possibly primary pulmonary large cell neuroendocrine carcinoma with aberrant CD45 expression.

When there is a malignancy of the lymph node, tissue samples should be handled properly so that pathologists can make a definitive diagnosis. However, with limited resources, general pathologists try to separate reactive conditions from the neoplastic process to provide a possible diagnosis of the type of malignancy found in the lymph node such as malignant lymphoma, metastatic carcinoma, metastatic melanoma, metastatic sarcoma, etc. Afterwards, all the slides, corresponding to tissue block(s), and a corresponding pathology report can be submitted to expert pathologists for further consultation.

Careful study of clinical history of any possible causes of lymphadenopathy warrants the exclusion of mimics for malignancy of the lymph node, including drug reactions and immunodeficiency states.

A review of the clinical history is paramount in clinical practice. It helps in making a clinical impression of the most likely malignancy of the lymph node. Moreover, it

TABLE 5. Panel of immunostaining commonly used in diagnosis of malignant lymphomas and leukemia (after only CD45 expression in immunostaining panels proposed in Tables 3 or 4).[5]

Marker	Positive in	Remarks
T-cell & NK cell lymphoma		
CD3	Normal T-cells, T-cell lymphoma, T-LBL, and NK/T-cell lymphoma	Usually membrane staining but cytoplasmic staining in NK/T-cell lymphoma or T lymphoblast
CD2, CD5, CD7	Same as CD3	Common T-cell markers; aberrant loss of any of them raises concern of neoplastic nature
CD4 & CD8	Helper & cytotoxic T-cells	Normal ratio of 2:1 in peripheral lymphoid tissue and blood; double negative (CD4-CD8-) or double positive (CD4+ CD8+) phenotype deems neoplastic
TCR-beta (betaF1)	Normal T-cells	90% of peripheral blood T-cells
TCR-gamma (GTCR)	Normal T-cells & primary cutaneous gamma-delta T-cell lymphoma	10% of peripheral blood T-cells
EMA	Positive up to 85% of ALK+ ALCL	Epithelial cells, some plasma cells
PD1, CXCL13, CD10, BCL6, ICOS-1, HGAL	TFH	Need at least 2 markers positive for diagnosis of AITL or nodal peripheral T-cell lymphoma of TFH phenotype
CD21 & CD23	FDC meshwork & hyperplasia	FDC hyperplasia for AITL
CD30	Activated/transformed lymphoid cells, HRS cell & ALCL	Normal activated/transformed lymphoid cells in T-cell zone
ALK	ALK+ ALCL	Nuclear, nuclear + cytoplasmic, or cytoplasmic pattern
B-cell lymphoma & Plasma cell neoplasm		
CD20	Normal & neoplastic mature B-cells including NLPHL	Membranous staining; may be faint positive in SLL/CLL or negative in some B-cell neoplasms; negative in PCM but may be occasional positive
CD10	Germinal center B-cell (both reactive & neoplastic), B-LBL	Positive in normal marrow B-cell precursors, normal & neoplastic bile canaliculi (CC), normal & neoplastic renal tubule (RCC); endometrial stromal sarcoma; non-specific staining in myeloid series in the marrow
CD5	Normal T-cell & neoplastic B-cells in SLL/CLL & MCL	
CD23	Normal B-cell, FDC & neoplastic B-cells in SLL/CLL	CD5+ CD23+ in SLL/CLL but CD5+ CD23- cyclin D1+ in MCL; CD21 is an adjunct marker for FDC
Cyclin D1	MCL, PCM, HCL	Usually negative in small B-cells in normal mantle layer of lymphoid follicle; also positive in epithelial cells in cell cycle
SOX11	MCL	Negative in leukemic phase of MCL; not a sensitive marker in practice
CD38, CD138	Normal & neoplastic plasma cell	CD38 not equal to CD138 & both not equal to immunoglobulin staining; CD138 also positive in epithelial cell

TABLE 5. Panel of immunostaining commonly used in diagnosis of malignant lymphomas and leukemia (after only CD45 expression in immunostaining panels proposed in Tables 3 or 4).[5] (Continued)

Marker	Positive in	Remarks
B-cell lymphoma & Plasma cell neoplasm		
Kappa & lambda Ig light chains	Evaluation of Ig light chain expression in plasma cells	Normal kappa to lambda ratio of 2:1; when either kappa or lambda more than the other at least 5 times raises the concern of monoclonal plasma cell population (plasmacytic differentiation)
IgG, IgA, IgM	Evaluation of Ig heavy chain expression in plasma cells	Usually IgG > IgA > IgM; usually performed when kappa+lambda less than estimated number of plasma cells (by morphology or CD38/CD138 immunostaining) in order to determine heavy chain disease
MUM1	Normal & neoplastic plasma cells, subset of DLBCL	Large B-cell lymphoma with *IRF4* rearrangement (need FISH)
ALK	ALK+ large B-cell lymphoma	
Classic Hodgkin lymphoma		
CD3	T-cells	Negative in HRS cell
CD15	Neutrophil & HRS cell	Sometimes only paranuclear positivity in HRS cell; less sensitive in formalin-fixed tissue
CD20	B-cells & NLPHL	Negative in typical CHL but variably positive in occasional HRS cells is also accepted
CD30	Activated/transformed lymphoid cells, HRS cell & ALCL	Normal activated/transformed lymphoid cells in T-cell zone
CD45	All types of lymphoid cells & up to 50% of ALCL	Negative in HRS cell
EMA	Epithelial cells, some plasma cells	Negative in HRS cell
MUM1	Positive in HRS cell	Normal & neoplastic plasma cells
PAX5	B lymphoblast to mature B-cell stage; faint positive in HRS cell	Negative in plasma cell
Leukemic infiltration		
CD34	Hematopoietic stem cells, blasts, endothelial cells	Less sensitive for blasts than flow cytometry
TdT	B-LBL, T-LBL	
CD33, MPO	Myeloblast	AML, M0: CD33+ CD34+ but MPO-
CD14, CD68	Monocyte/promonocyte/monoblast	Immature morphology
CD99	Immature hematopoietic cell	Express on normal early thymocyte
CD117	Myeloid & erythroid precursors	Express on normal mast cells
CD123	BPDCN	Treated as ALL

Abbreviations: AITL: angioimmunoblastic T-cell lymphoma, ALCL: anaplastic large cell lymphoma, ALL: acute lymphoblastic leukemia, AML, M0: Minimally differentiated acute myeloid leukemia, B-LBL: B-lymphoblastic lymphoma/leukemia, BPDCN: blastic plasmacytoid dendritic cell neoplasm, CC: cholangiocarcinoma, FDC: follicular dendritic cell, FISH: fluorescence in situ hybridization, HCL: hairy cell leukemia, HRS: Hodgkin-Reed-Sternberg, Ig: immunoglobulin, MCL: mantle cell lymphoma, NLPHL: nodular lymphocyte predominant Hodgkin lymphoma, PCM: plasma cell myeloma (multiple myeloma), RCC: renal cell carcinoma, SLL/CLL: small lymphocytic lymphoma/chronic lymphocytic leukemia, TFH: T follicular helper, T-LBL: T-lymphoblastic lymphoma/leukemia

TABLE 6. Panel of immunostaining commonly used in diagnosis of carcinoma and epithelioid variant of sarcoma (after only AE1/AE3 expression in immunostaining panels proposed in Tables 3 or 4)[15,31]

Marker	Positive in	Remarks
Adenocarcinoma		
CK7 & CK20	CK7+/CK20+: Pancreas, bile duct, stomach, urinary bladder	CK7+/CK20-: Breast, endometrium, ovary, lung, thyroid (also positive in malignant mesothelioma)
	CK20+/CK7-: Colorectum, Merkel cell carcinoma & occasional upper GI	CK7-/CK20-: Adrenal cortical carcinoma, prostatic carcinoma, HCC, RCC, neuroendocrine carcinoma of lung & GI tract
CDX2	Colorectum	
GATA-3	Breast	Also positive in UC, pheochromocytoma, paraganglioma, choriocarcinoma, malignant mesothelioma
Hepar 1, Glypican-3, Arginase-1	HCC	
NKX3.1	Prostate, breast	Positive in invasive lobular carcinoma
PAX8	Endometrium, ovary, thyroid, RCC	PAX8+ CD45+ AE1/AE3- DLBCL
SATB2	Colorectum	Also positive in neuroendocrine carcinoma, osteosarcoma, BCOR-rearranged sarcoma
TTF-1	Lung, thyroid	
Squamous cell carcinoma		
CK5/6	+	Also positive in mesothelioma, BCC, UC
p40	+	Also positive in BCC, UC
p63	+	p63+ CD45+ AE1/AE3- DLBCL
Neuroendocrine carcinoma		
CD56	+	Also + in other types of cancer
CK8/18	+	
Chromogranin A	+	
Synaptophysin	+	
Ki-67	>20%	<20% in neuroendocrine tumor
Epithelioid variant of sarcomas		
CD34, ERG	Epithelioid Angiosarcoma	Loss of SDH subunit B
CD117, DOG1	SDH-deficient GIST	
Desmin, myogenin	Epithelioid RMS	
INI-1 loss	ES, epithelioid MPNST	Also positive in melanoma & LCH
S100	Epithelioid MPNST	Also positive in melanoma
SOX10	Clear cell sarcoma	Also positive in ES, ESS, EWS, MPNST, schwannoma, and SFT
TLE-1	Synovial sarcoma	

Abbreviations: BCC: basal cell carcinoma, DLBCL: Diffuse large B-cell lymphoma, ES: epithelioid sarcoma, ESS: endometrial stromal sarcoma, EWS: Ewing sarcoma, HCC: hepatocellular carcinoma, LCH: Langerhans cell histiocytosis, MPNST: malignant peripheral nerve sheath tumor, RCC: renal cell carcinoma, RMS: rhabdomyosarcoma, SCC: squamous cell carcinoma, SDH-deficient GIST: succinate dehydrogenase-deficient gastrointestinal tumor, UC: Urothelial carcinoma

TABLE 7. Panel of immunostaining commonly used in diagnosis of malignant melanoma and sarcoma (after only S100 expression in immunostaining panels proposed in Tables 3 or 4)[31]

Marker	Positive in	Remarks
Malignant melanoma		
SOX10		Also positive in clear cell sarcoma & epithelioid MPNST
HMB45		Also positive in angiomyolipoma
Melan A		Also positive in adrenal cortical carcinoma & stromal sex cord tumor
Sarcoma		
See epithelioid variant of sarcoma in Table 6		

Abbreviation: MPNST: malignant peripheral nerve sheath tumor

helps to exclude reactive conditions, infectious processes, and abnormal immune reactions, especially drug reactions and immunodeficiency states, as discussed earlier. The location of the enlarged lymph node implies possible causes such as metastatic CA breast in the axillary lymph node in female patients, metastatic nasopharyngeal carcinoma in level II (upper jugular group) of cervical lymph nodes, metastatic CA thyroid in level IVa (lower jugular group), IVb (medial supraclavicular group), or VIb (deeper pre-laryngeal/pre-tracheal group) of cervical lymph nodes, metastatic CA stomach in left supraclavicular lymph node, and metastatic melanoma in inguinal lymph node. However, at times, unexpected metastatic tumors are observed in unusual locations such as the CA prostate with metastasis to the cervical lymph node.[25] However, the most difficult case is cancer of unknown primary (CUP) in clinical practice. CUP rarely presents in lymph node only (LNCUP), according to a series from MD Anderson Cancer Center. LNCUP has better clinical outcomes than CUP in general or CUP with predominant bone disease.[26] While axillary LNCUP in women is treated as CA breast with axillary lymph node metastasis, histologic type of adenocarcinoma seems to have better clinical outcomes than other histologic types.[27] In a LNCUP case with only one lymph node group positive for metastatic carcinoma, it may be possible to track the primary site according to sentinel node theory, but it is limited to one organ with one direction of lymphatic drainage. It cannot be applied to supraclavicular lymph nodes that receive lymphatic drainage from many organs or lymph node metastasis with more than two directions of lymphatic drainage.[28]

Adequate cell or tissue samples will allow pathologists to work efficiently and master the multimodality approach under good clinical collaboration.

General practitioners, especially ones who perform tissue biopsy, can help by paying attention to the details of the tissue obtained. Most cellular tumors have soft to firm light brown tissue texture with or without accompanying hemorrhaging or necrosis. White tough fibrous tissue should be avoided and if the tissue biopsy looks like that, doing another biopsy to obtain more representative tissues is a must or the pathology report will come back as "fibrotic tissue obtained, please do another biopsy." In the past, pathologists preferred complete excision of the enlarged lymph node so that a complete evaluation could be performed. Fine needle aspiration (FNA) of the lymph node is not recommended in suspected cases of malignant lymphoma. However, technical advances have provided options for improvements such as a FNA accompanied by flow cytometry for lymphoma panel. However, core needle biopsy is widely accepted in practice but a large panel of immunostaining is required to obtain more information to compensate the limited histologic evaluation.

Effective communication between pathologists and physicians regarding relevant laboratory investigations should make it easier to make a definitive diagnosis for the type of malignancy

There is no doubt that effective communication between pathologists and physicians is the best way to achieve a definitive diagnosis of a malignancy based

on relevant laboratory investigations in addition to good clinical history as mentioned earlier. Even better access via a laboratory information system (LIS) allows pathologists to find relevant laboratory results easier, while a discussion of the case with attending physicians usually reveals important issues of concern about the diagnosis of tumor type. However, in a small number of cases, it is very difficult to acquire a definitive definite diagnosis when the tumor cells do not differentiate well (undifferentiated tumor).

Pathology of tissue sample handling and diagnostic process

The way pathologists handle cell or tissue samples by conventional morphologic evaluation and panels of immunostaining provides insight to general practitioners to understand the diagnostic process in pathology. In terms of quality assurance, laboratory work involves pre-analytic, analytic, and post-analytic phases. The pre-analytic phase focuses on specimen collection. In this step, the general practitioner needs to know how to obtain the specimen correctly and choose the appropriate test. Regarding pathologic diagnosis, FNA, core needle biopsy, incisional biopsy or excision of the enlarged lymph node should be selected based on clinical information as well as accessibility to the lesion of concern. FNA of the cervical lymph node with a clinical concern of nasopharyngeal carcinoma or cancer of the head and neck region is deemed appropriate. In contrast, this process is not rewarding for malignant lymphoma unless a flow cytometry of fresh samples from FNA is performed at the same time. So, FNA is not recommended in a suspected case of malignant lymphoma where flow cytometry is not available. Instead, excision of the enlarged lymph node is recommended. In places with limited resources, imprints of a fresh cut surface of the lymph node provide cytologic features of lymphoma cells. A good cytotechnologist or even a well-trained hematologist can make a definitive diagnosis from well prepared lymph node imprints, such as Burkitt lymphoma.

Another important issue in the pre-analytics phase is the quality of tissue sample. It should be handled properly so that the slides stained with hematoxylin and eosin (H&E) can be examined by pathologists without interference in the interpretation process. The tissue sample should be fixed in a good volume of neutral buffered formalin (10 times the volume of the tissue sample) for at least two to three hours prior to tissue processing in the laboratory. If core needle biopsy of the lymph node is performed, practitioners must make sure that a proper lymph node tissue is obtained. The tissue should be soft to firm and light brown. If the tissue is tough white fibrous or pale yellow necrotic, more tissue cores are needed. In case of necrotic tissue, microbiologic studies should be considered as well.

The analytics phase depends on a pathologist's performance. Good quality H&E-stained slides should allow pathologists to gather relevant microscopic findings to make a diagnosis. Recognition of particular patterns should lead to a list of differential diagnosis. Ultimately, it depends on a pathologist's knowledge of histology, pathology, training background, technical skills, perception, and memory.[29] Then, all clinical and pathological findings are analyzed using the pathologist's knowledge and experience to provide an interpretation of the lymph node biopsy. The diagnosis could be a straightforward textbook case for any malignancy of the lymph node, however, in a problematic case, additional clinical and laboratory information as well as clinical impression must be considered. Special stains, histochemistry, and immunostaining are requested in order to gather more information to decide the nature of tumor cells. At this point, authorization and expenses can take time, depending on the health care system. If the chain of analysis is allowed to flow freely, an experienced pathologist may handle the case efficiently and provide a diagnosis within 24 or 48 hours, based on histologic evaluation, immunostaining, and/or a number of in situ hybridization (ISH) techniques such as ISH for EBV-encoded small RNA (EBER ISH). In some institutes, a double sign-out system will ask two pathologists to look at the case of malignancy diagnosed for the first time in order to confirm a diagnosis before releasing the pathology report.[30]

The post-analytic phase depends on the attending physician determining whether the pathologic diagnosis of any type of the malignancy of the lymph node is clinically relevant. In some institutes, before releasing the pathology report, the pathologist and the physician who submits the tissue sample have already discussed the case. Any physician who later gets involved in patient management has a right to challenge the diagnosis when it is not relevant to the clinical information. For example, the pathologist gives a diagnosis of CHL but there are a number of clinical findings suggest NHL. The pathologist should be notified and asked to review the case. Moreover, in any malignancy diagnosed from other hospitals, all pathologic materials (slides, corresponding tissue blocks, and pathology report) should be reviewed by an experienced pathologist to confirm the diagnosis before starting any specific treatment.

CONCLUSION

By understanding the basic principles and the types of malignancy found in the lymph node, a careful study of the clinical history, adequate cell or tissue samples from the lymph node, good clinical collaboration with effective communication between pathologists and physicians, and mastery in conventional morphologic evaluation along with an appropriate panel of immunostaining, general practitioners and pathologists could make the diagnostic process easier in order to diagnose the malignancy of the lymph node in most cases.

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