Getting the Diagnosis Right in NHL: Role of Immunohistochemistry and Molecular Diagnostic Testing

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Abstract

Challenges in diagnosing lymphoid neoplasms include their complex heterogeneity and the fact that approximately 40 or more diseases exist. Immunophenotyping and molecular diagnostics have made important contributions to precise and accurate diagnoses. Strong interactions with clinical colleagues and meticulous attention at the microscope by expert hematopathologists are important in making a correct diagnosis. Awareness of the literature and interactions with research colleagues, including clinical, basic, and translational scientists, have expanded understanding of these complex diseases, providing prognostic information that can ultimately assist in appropriate clinical management of patients or development of new targeted therapies. This article reviews recent published immunophenotypic diagnostic and biomarker studies, discusses molecular diagnostic expression profiling studies of the more common entities encountered in daily clinical practice, and references published summaries from the combined Society of Hematopathology and European Association for Haematopathology Workshops. A precise lymphoma diagnosis therefore involves integration of clinical information, morphologic architectural and cytologic patterns, immunohistochemistry, cytogenetics, and molecular biology, to ultimately allow identification of specific diseases, thereby implying prognostic significance and potential therapeutic targets. (JNCCN 2008;6:422–427)

Key Words

Lymphoma, diagnoses, immunohistochemistry, molecular

Lymphomas represent neoplastic proliferations of the immune system, which is complex and involves various cells of origin, differentiation processes, activation pathways, and the topographic diversity of tissues, including tissue compartmentalization and microenvironment interactions. Pathogenetic mechanisms are heterogeneous, involving different molecular and genetic alterations, infectious agents, immunosuppression, and possible occupational exposures. A precise and accurate lymphoma diagnosis requires recognizing specific features of a disease, particular clinical manifestations and disease evolution, and a distinctive pathogenesis with prognostic implications that ultimately influences the clinical management, and enables identification of optimal therapeutic strategies. It also requires making a differential diagnosis among analogous entities.

Immunophenotyping has contributed importantly to the diagnosis of lymphomas. Although some subtypes, such as follicular lymphoma, can be accurately diagnosed based on morphology alone, immunophenotyping is important for most patients, increasing diagnostic accuracy in diffuse large B-cell lymphoma from 73% to 87%, and anaplastic large T/null cell lymphoma and other peripheral T-cell lymphomas from 41% to 46% up to 85% to 86% after immunophenotyping.¹

Diffuse large B-cell lymphoma is the most common lymphoma subtype but is clinically heterogeneous, with up to 13 subtypes listed in the 2008 draft of the World Health Organization (WHO) International Classification of Diseases. Recently, anatomic site and expression profiling data have been used as parameters for classification, such as primary cutaneous large B-cell lymphoma, primary mediastinal large B-cell lymphoma (PMLBCL), and diffuse large B-cell lymphoma (DLBCL) of immunoprivileged sites, such as the central nervous system and...
testes. Primary cutaneous large B-cell lymphoma affecting the head and neck have a germinal center cell profile and excellent prognosis, whereas the leg-type lymphoma has an activated B-cell profile and a poor prognosis. PMLBCL is a distinct entity primarily affecting young women with a bulky mediastinal mass and frequent extrathoracic relapses. Immunophenotyping shows discordant CD79A expression and absence of immunoglobulin expression. Molecular genetic alterations include gains in 9p (JAK2) and 2p13 (REL), and SOCS1 gene mutations. PMLBCL shows overlapping clinical, morphologic, and genetic features with Hodgkin lymphoma that can distinguish it from other DLBCLs, including TRAF1 and c-REL expression, BCL6 mutations and expression, and suggestion of frequent loss of major histocompatibility complex (class II) proteins but increased expression of B-cell signaling molecule. Mediastinal grey zone lymphomas may represent the histologic link between the morphologic and genetic spectrums of PMLBCL and Hodgkin lymphoma. The B-cell transcription factors also are helpful in distinguishing a CD30-positive DLBCL from classical Hodgkin lymphoma.

Much recent activity has focused on developing biologic prognostic indicators, initially involving gene expression profiling and then immunohistochemical biomarkers, such as cell cycle regulatory molecules (p53, p27, cyclin D2, D3, MIB1), apoptosis-related molecules (BCL2, survivin), B-cell differentiation molecules (BCL6, CD10, HGL, CD5, FOXP-1, CD21s), and adhesion-related molecules (ICAM, sCD44). A 6-gene model, including LMO2, BCL6, FN1, CCND2, SCYA3, and BCL2, strongly predicted for survival in patients with DLBCL treated with anthracycline-based regimens. Recently a similar 6-gene model using a paraffin-based assay predicted for survival in patients with DLBCL treated with R-CHOP (cyclophosphamide, doxorubicin, Oncovin, and prednisone with rituxan) therapy, showing promise with application to clinical samples. Recent genetic profiling studies also indicate specific genes and possible immunophenotypic marker panels to distinguish Burkitt lymphoma, DLBCL, and B-cell lymphoma with features intermediate between DLBCL and Burkitt lymphoma.

Numerous immunohistochemical surrogate prognostic marker studies in DLBCL used in combination with the clinical international prognostic indices to help predict how patients will respond to chemotherapy has led to varying results. An international collaborative project recently recommended the importance of harmonization of techniques, uniformity of scoring criteria, and centralized consensus review in multicenter clinical trials before proceeding to broad clinical application. Other genetic and murine modeling studies have also identified possible new therapeutic targets.

The prototypic case of follicular lymphoma shows a follicular proliferation of centrocytes and centroblasts associated with follicular dendritic cells. Tumor cells are positive for CD20, CD19, CD79a, IgG, IgM, IgD, CD10, BCL2, and BCL6, but negative for CD5. Hallmark genetics shows a JH/BCL2 gene rearrangement, t(14;18), and somatic mutations of VH genes. Most patients are adults presenting with advanced-stage disease with an indolent clinical course but are incurable. Cytologic grading of follicular lymphoma among pathologists lacks reproducibility; however, most studies show a difference in natural history and overall survival among the different cytologic grades and diffuse growth pattern. Nevertheless, the long-term significance of grading is still controversial. The proliferation marker MIB-1 or Ki-67 may be a better prognostic indicator than cytologic grade. Variable CD10 expression and positive MUM1 are seen in higher cytologic grades, whereas BCL6 and CD10 are associated with longer overall survival and time-to-treatment-failure in follicular lymphoma. CD5- and CD23-positive follicular lymphomas have been described, but the significance is not known. BCL2 is helpful in distinguishing reactive follicular hyperplasia or partial in situ localization. The microenvironment, such as T-cell or macrophage infiltration, also indicates prognostic significance (i.e., increased monocytes indicates a poor prognosis), as shown with gene expression studies and subsequent immunohistochemistry on tissue microarrays. Pediatric follicular lymphoma has been recognized as a distinct entity and must be distinguished from florid follicular hyperplasia. Primary follicular lymphoma involving the duodenum, unrecognized until recently, reflects how anatomic location can be a parameter for classification.

The prototypic case of mantle cell lymphoma is composed of small centrocyte-like cells, with the blastoid variant having an aggressive clinical course. Tumor cells are positive for CD20, CD79a, slgM/IgG, CD5, and cyclin D1, but negative for CD23, with CD10 showing
the t(11;14). Adult men are mainly affected, with a median survival of 3 to 4 years. Overall survival can be stratified by MIB-1 or Ki-67.17 Gene expression studies showed variable expression in proliferation signatures, but it was found to correlate with overall survival and morphologic subtypes, such as the blastoid, classical, small cell, or pleomorphic variants.28 Cyclin D1-negative, CD5-positive, and CD23-negative cases exist that express cyclin D2 or D329 and share similar secondary genetic aberrations.10 CD5-negative cases are also not uncommon, ranging from 5% to 27% in reported series.31 Based on active investigation of the cell cycle, apoptotic death, and DNA damage-related pathways, several agents are available in preclinical and phase I/II clinical trials.32 Murine models will be helpful in developing and exploring additional therapeutics.33-35

The prototypic case of chronic lymphocytic leukemia/small lymphocytic lymphoma is dimly positive for CD20 and surface immunoglobulin heavy chain IgM and IgD, with kappa light chains greater than lambda light chains, and positive for CD5 and CD23 but negative for CD10, BCL6, and cyclin D1. The presence or absence of somatic mutations in immunoglobulin variable heavy chain genes distinguishes 2 subgroups with different clinical outcomes.36 Expression profiling has shown Zap-70 to be highly expressed in unmutated chronic lymphocytic leukemia.37 Furthermore, it is a reliable surrogate marker for mutational status, predicts for poor outcome,38,39 and is also expressed in other lymphomas.40 Bone marrow biopsies can also be stained for Zap-70 using immunohistochemistry.41 Peripheral blood lymphocytosis of a mature B-cell infiltrate can be analyzed with flow cytometry or the bone marrow biopsy analyzed with the low-grade B-cell lymphoma panel, including CD20, CD3, CD5, CD23, CD10, BCL2, CD43, and BCL6, to definitively rule out leukemic infiltration by chronic lymphocytic lymphoma, follicular lymphoma, or marginal zone lymphoma (MZL). To definitively rule out hairy cell leukemia, annexin, c-TRAP, and CD20 can also be performed on bone marrow sections using immunohistochemistry.42

Extranodal mucosa-associated lymphoid tissue (MALT) lymphoma shows reactive germinal centers with lymphoepithelial lesions and centocyte-like cells, presence of light-chain restriction, absence of CD10/CD5 expression, and characteristic chromosomal translocations detected with fluorescence in situ hybridization. Associations with anatomic localization, cytogenetic alterations, dysregulated genes, and infectious origins have also been suggested and recognized in this entity.43,44 However, classic cytomorphology is not always present, polymerase chain reaction analysis of small biopsies may produce false-positivity in detecting immunoglobulin H clonality, and distinction from lymphoplasmacytic lymphoma may be difficult. For nodal MZL, adjacent mucosal disease must be excluded; B-cells do not express CD5 and CD10; characteristic translocations found in MALT are absent; and this entity is almost a diagnosis of exclusion. The immunoglobulin superfamily receptor translocation (IRTA-1) gene seems to be a valuable marker for nodal and extranodal MZL.45,46 The current 2008 draft of the WHO International Classification of Diseases also proposes pediatric MZL as a separate category.47,48

Distinguishing MZL from marginal zone hyperplasia through coexpression of BCL2 and lack of light-chain expression is also important.44 Distinguishing MZL from lymphoplasmacytic lymphoma or CD20-positive plasma cell neoplasm is also challenging, because immunohistochemistry and flow cytometry show that both entities lack CD5 and CD1.49 Presence of mast cells in bone marrow suggests a lymphoplasmacytic lymphoma over MZL. Immunohistochemistry assays for kappa, lambda, CD138, and CD20 proteins are routine in the authors’ laboratory, and in situ hybridization for kappa and lambda on bone marrows and lymph nodes are performed for unclear cases.

Splenic MZL, another distinct clinicopathologic entity, has a biphasic morphology, predominantly white pulp involvement, and an uncommon red pulp pattern, with neoplastic B-cells failing to express CD5, CD10, CD23, and CD43; occasional cases express CD5 and some express weak CD23. Frequent peripheral blood and intrasinusoidal bone marrow involvement associated with adhesion molecules has been suggested.50 Diagnosis without splenectomy can be difficult. Up to 70% of cases show a del7q31–32 and del7q21 suggesting CDK6 gene deregulation.51 Biologic and clinical prognostic indicators may stratify patients according to risk and suggest specific therapies based on treatment algorithms.52

Immunophenotyping increases the accuracy of diagnosing anaplastic large cell lymphoma (ALCL) among other peripheral T-cell lymphomas from 41% to 46% up to 85% to 86%.1 Anaplastic lymphoma kinase (ALK)-positive ALCL has become a relatively...
well-defined entity at the histologic, immunophenotypic, and molecular levels. The availability of ALK immunostaining has allowed pathologists to recognize the wide histologic spectrum and unusual clinical manifestations of this disease. Rare cases can mimic the nodular sclerosis variant of classic Hodgkin lymphoma.\(^\text{51}\) Currently, no clear consensus exists regarding ALK-negative ALCL, although the predominant opinion is that these neoplasms are not a distinct entity at the immunophenotypic or molecular level, as discussed in session 8 of the 2005 Society of Hematopathology/European Association for Haematopathology Workshop, which was devoted to ALCL.\(^\text{54}\) Additional markers are needed to predict which cases of cutaneous ALCL will disseminate and to distinguish systemic ALK-negative ALCL involving skin from cutaneous ALCL.\(^\text{54,55}\)

One study showed that an aberrant phenotype with frequent loss of CD5 and CD7 was a typical finding for unspecified and angioimmunoblastic peripheral T-cell lymphoma (PTCL; unspecified [PTCL/U] and PTCL/angioimmunoblastic T-cell lymphoma [AILT], respectively) and that an MIB-1 (Ki-67) proliferation rate greater than 80% was prognostically relevant and therefore integrated in a new predictive score that included age (> 60 years), high lactate dehydrogenase, poor performance status, and Ki-67 greater than 80%.\(^\text{56}\)

Most cases in this study were positive for CD2 (100%), CD3 (86%), and T-cell receptor βF1 (97%), whereas 55% of samples were either double-positive or double-negative for CD4 and CD8. CD10 was detected in 2 of 143 samples of PTCL/U compared with 17 of 43 PTCLs/AILT, confirming expression as previously reported.\(^\text{57}\) AILT expresses CXCL13, suggesting a follicular helper T-cell derivation.\(^\text{58}\) Discussions of diagnostic approaches and pitfalls using immunophenotyping in cutaneous T-cell lymphomas, natural killer cell lymphomas, enteropathy-type T-cell lymphoma, and hepatosplenic and gamma/delta T-cell lymphomas, and new information about disease pathogenesis from presented cases at the 2005 Hematopathology/European Association for Haematopathology Workshop are presented elsewhere.\(^\text{59–63}\)

In summary, Table 1 lists brief take-home messages for the more commonly encountered entities. Future perspectives and challenges in lymphoma diagnosis include the development of new data and concepts that are important for future efforts to define new disease entities, such as anatomic location as a parameter for classification, differentiation plasticity of hematologic cells, and new technologies. The question of whether lymphoma entities are stable is intriguing, as shown by transdifferentiation and the dynamics of lymphoid populations.\(^\text{64}\) Tumor host interactions may also play an important role. Daily clinical practice shows that lymphoma entities are not necessarily pure, as shown by composite lymphomas present in the same tissue biopsy and discordant sequential lymphomas.

### Table 1 Brief Take-Home Messages and Future Challenges

| Diffuse large B-cell lymphoma: despite recent marked advances in gene expression profiling and numerous surrogate immunohistochemical biomarker studies, analyses in the R-CHOP (cyclophosphamide, doxorubicin, Oncovin, and prednisone) setting with the Revised International Prognostic Index and centralized consensus review in multicenter clinical trials have been suggested before broad clinical use can affect patient management decisions | Diffuse large B-cell lymphoma: despite recent marked advances in gene expression profiling and numerous surrogate immunohistochemical biomarker studies, analyses in the R-CHOP (cyclophosphamide, doxorubicin, Oncovin, and prednisone) setting with the Revised International Prognostic Index and centralized consensus review in multicenter clinical trials have been suggested before broad clinical use can affect patient management decisions |
| Primary mediastinal large B-cell lymphoma: overlap with Hodgkin lymphoma by gene expression profiling, and use of TRAF1 and cREL are recognized | Primary mediastinal large B-cell lymphoma: overlap with Hodgkin lymphoma by gene expression profiling, and use of TRAF1 and cREL are recognized |
| Follicular lymphoma: biomarkers for improved prediction of grade such as MIB1 over cytologic grading, surrogate biomarkers for microenvironment molecules, and pediatric variants have recently been recognized | Follicular lymphoma: biomarkers for improved prediction of grade such as MIB1 over cytologic grading, surrogate biomarkers for microenvironment molecules, and pediatric variants have recently been recognized |
| Mantle cell lymphoma: cyclin D2 and D3 positive cases, and morphologic variants are recognized | Mantle cell lymphoma: cyclin D2 and D3 positive cases, and morphologic variants are recognized |
| Hairy cell lymphoma: annexin and cTRAP can help to distinguish atypical hairy cell lymphoma and splenic marginal zone lymphoma | Hairy cell lymphoma: annexin and cTRAP can help to distinguish atypical hairy cell lymphoma and splenic marginal zone lymphoma |
| Marginal zone lymphoma: IRTA1 for nodal and extranodal types; site specific characteristic translocations; pediatric variants and use of BCL2 and IGH to distinguish marginal zone hyperplasia are recognized | Marginal zone lymphoma: IRTA1 for nodal and extranodal types; site specific characteristic translocations; pediatric variants and use of BCL2 and IGH to distinguish marginal zone hyperplasia are recognized |
| Lymphoplasmacytic lymphoma: CD20, P63, K, L, mast cells, serum heavy chain, and clinical correlation can be helpful in distinguishing marginal zone lymphoma with plasmacytic differentiation | Lymphoplasmacytic lymphoma: CD20, P63, K, L, mast cells, serum heavy chain, and clinical correlation can be helpful in distinguishing marginal zone lymphoma with plasmacytic differentiation |
| Anaplastic large-cell lymphoma: ALK-positive and -negative cases; variant translocations with various cellular localizations; rare cases that can mimic nodular sclerosis variant of Hodgkin lymphoma; and molecules that predict cutaneous versus systemic origin will be helpful to recognize | Anaplastic large-cell lymphoma: ALK-positive and -negative cases; variant translocations with various cellular localizations; rare cases that can mimic nodular sclerosis variant of Hodgkin lymphoma; and molecules that predict cutaneous versus systemic origin will be helpful to recognize |
occurring in the same patient, whether 2 clonally related or unrelated low-grade B-cell lymphomas, a B- and T-cell lymphoma, or Hodgkin lymphoma and non-Hodgkin’s lymphoma. In summary, a precise and accurate diagnosis requires a strong knowledge of lymphoma entities, experience, use of all available multidisciplinary resources, and ultimate integration of all data by the pathologist to help clinicians provide optimal appropriate clinical therapy.

References

Immunohistochemistry and Molecular Diagnostic Testing in NHL


