Evolution of Lymphoma Diagnosis in the Era of Personalized Medicine

A Marriage of Pathology and Genomics for Clinical Practice

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The historical basis for the classification of lymphoma and other neoplasms is based on histopathology. The first use of the term lymphosarcoma is attributed to Rudolf Virchow in 1863. In 1898 and 1902, Carl Sternberg and Dorothy Reed independently described the characteristic binucleate and multinucleate giant cells that came to be known as Reed-Sternberg cells. The contemporary classification of lymphomas began with Rappaport, who outlined his concepts in the Armed Forces Institute of Pathology fascicle published in 1966. He stratified lymphomas according to cell types and architectural patterns and proposed the term nodular lymphoma. However, he did not attribute this pattern to normal lymphoid follicles. Subsequently, Karl Lennert recognized common cytologic features between normal germinal centers and lymphomas with a follicular growth pattern. He coined the terms centrocyte and centroblast, which we still employ today for the principal cells comprising follicular lymphoma (FL). The immunologic revolution began in earnest in the 1970s, when many authors sought to relate lymphomas to the cells of the normal immune system. In 1974, Jaffe et al identified that the cells of nodular lymphoma, like the cells of the normal germinal center, expressed complement receptors, confirming their follicle center derivation. However, progress in what we still call diffuse large B-cell lymphoma (DLBCL) proceeded more slowly. Although early studies identified

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The Gold-Headed Cane Award is the oldest and most prestigious award given by American Society for Investigative Pathology (ASIP). This award recognizes significant long-term (lifetime) contributions to experimental pathology research, outstanding teaching, general excellence in the discipline, demonstrated leadership in the field of pathology, as well as engagement in the activities of the Society. Elaine S. Jaffe, recipient of the ASIP 2022 Gold-Headed Cane Award, delivered a lecture entitled “Classification of Lymphoma in the Modern Era: A Marriage of Pathology and Genomics” on April 3, 2022, at the Experimental Biology meeting in Philadelphia, PA.
that many of these more aggressive tumors were B-cell derived, clinically relevant subdivisions of this lymphoma subtype are a much more recent advance.

Today, we are entering an era of personalized medicine, in which traditional diagnostic approaches are being combined with genomic studies to advance our understanding of neoplastic disease. This approach was embraced by the Revised European-American Lymphoma classification, and subsequently adopted for the World Health Organization classification. FL and DLBCL were historically viewed as single diseases and are the most common subtypes of lymphoma on a worldwide basis. However, more recent genomic studies illuminated significant diversity, even among morphologically similar lesions, and these advances have impacted clinical diagnosis and management.

Follicular Lymphoma

In simplest terms, FL is a B-cell malignancy that mimics normal follicles histologically and phenotypically. However, follicle center neoplasms do not constitute a single disease, and they represent several distinct entities differing in their biology, clinical behavior, and management. Classic FL was one of the first lymphoma subtypes to be associated with a recurrent genomic aberration, the $BCL2$ rearrangement ($BCL2\text{R}$). This form of FL arises from bone marrow precursor cells that acquire the t(14;18) during early stages of Ig gene rearrangement (Figure 1). This translocation represents the founder event, and clinically significant disease results from a successive accumulation of complementary genetic and epigenetic alterations. In FL, cells termed clonal progenitor cells undergo multiple cycles of germinal center reentry, with clonal expansion, genomic instability, and ultimate dissemination. Early studies found cells carrying the t(14;18), termed FL-like B cells, in healthy adults. Such cells can be seen in approximately 70% of individuals aged >50 years. However, the risk of subsequent FL is exceedingly low in this setting, in the range of 0.01%.

A histologic counterpart of FL-like B cells was recognized in reactive lymph nodes, initially termed in situ FL, and it is now designated as in situ follicular neoplasia, due to its low risk of clinical progression, which is <5%. Duodenal-type FL is a closely related incipient form of FL. By array comparative genomic hybridization, both of these early lesions have a low level of genomic aberrations beyond the $BCL2$ translocation. However, they may show mutations in $CREBBP$, $EZH2$, and $TNFRSF14$. Mutations in $KMT2D$ appear to be a later event, associated with an increased risk of progression.

Relapses in FL occur both by linear and divergent evolution. Histologic progression over time is an intrinsic part of FL biology. A high proportion of patients with FL will undergo histologic progression and transformation over their clinical course. The disease can acquire remarkably
different phenotypes, and it may even show evidence of lineage switch. The most common form of histologic progression is to DLBCL. Common secondary events associated with evolution to DLBCL include mutations in *TP53* and cyclin-dependent kinase inhibitors. Acquired translocation of *MYC* is seen in some FL, commonly termed double-hit lymphoma, and exhibits an aggressive clinical course.

These lesions may show expression of terminal transferase but lack other features indicative of B-lymphoblastic lymphoma/leukemia. Other more infrequent forms of histologic progression, also clonally related to the original FL, include classic Hodgkin lymphoma and histiocytic sarcoma. Secondary histiocytic sarcomas share some of the molecular alterations of FL but also show evidence of evolution with acquisition of new mutations characteristic of primary histiocytic sarcoma, such as those in the rat sarcoma virus/rapidly accelerated fibrosarcoma (RAS/RAF)/mitogen-activated protein kinase pathway.

The optimal grading scheme for FL has been challenging. Historically, the World Health Organization classification has used a traditional system in which low-grade lesions, composed of a majority of centrocytes, are designated as grade 1 or 2. Cases with >15 centroblasts per high-power field are designated as grade 3A, although it has been unclear whether these patients require more aggressive therapy or are at a higher risk for progression to DLBCL. The recently proposed World Health Organization fifth edition took the somewhat controversial step of suggesting that grading of FL was no longer required for routine clinical practice.

Recently, Drevet et al. used a multistep process to define the genetic signature of FL cases that might be at greater risk for transformation. Two FL subtypes could be identified by this approach, with differences in mutation signatures, mutated gene frequencies, gene expression, and translocations. Cases designated as constrained FL had a markedly reduced risk of transformation compared with those designated as DLBCL-like FL. Interestingly, the DLBCL-like FL profile was more often encountered in cases designated grade 3A. In the future, genetic profiling is likely to be more informative than histologic grading, but it will take time for these approaches to be implemented in clinical practice.

**Other Follicle Center B-Cell Neoplasms**

There has been general agreement that FL grade 3B, composed of a pure population of centroblasts, is biologically closely linked to DLBCL and is negative for *BCL2* R in most cases. However, in clinical practice, the distinction of grade 3A from grade 3B is not always easy or reproducible. The International Consensus Classification recommends that genetic studies, including fluorescence in situ hybridization for *BCL2* R, be routinely employed in this setting, and that presence of *BCL2* R strongly favors FL grade 3A over 3B, whereas FL grade 3B usually differs from classic FL in its genotype and phenotype. In addition to lacking the translocation that defines classic FL, these cases are often negative for CD10, and may express aberrations involving *BCL6*.

In addition, other forms of B-cell lymphoma either manifest a follicular growth pattern or exhibit a phenotype suggestive of follicle center derivation that should be recognized as distinct from classic FL. Most of these lesions exhibit major clinical differences from FL and all lack *BCL2* R. One subtype, newly recognized as a provisional entity in the International Consensus Classification, is *BCL2* R−negative, CD23+ follicle center lymphoma. Most patients present with low-stage disease, often with involvement of inguinal lymph nodes. The growth pattern can be follicular or diffuse. These cases have a characteristic genomic profile with frequent *STAT6* mutation, usually correlating with strong expression of CD23.

Several other follicle center–derived neoplasms occur predominantly in children and young adults and are recognized as separate diseases. These include pediatric-type follicular lymphoma, testicular follicular lymphoma, and IRF4-rearranged large B-cell lymphoma. Pediatric-type follicular lymphoma is a disease most common in young boys, usually presenting with isolated cervical lymphadenopathy. It has a low level of genomic complexity, with frequent aberrations involving *MAP2K1* and 1p36/*TNFRSF14*. A recurrent loss-of-function mutation in *IRF8*, a tumor suppressor gene, was also reported more recently. Clinical management is conservative, with most patients cured by local excision. Evidence of marginal zone differentiation is common in these lesions, with nodal marginal zone lymphomas occurring in children being part of the same entity.

The molecular pathogenesis of testicular follicular lymphoma is less well understood, but most patients are cured by simple orchietomy. *IRF4*-rearranged large B-cell lymphoma presents equally in males and females, most often involving the Waldeyer ring, and usually has at least a focal follicular growth pattern. Despite the large cell size, sometimes resembling DLBCL, it has an excellent prognosis, and likely requires less intensive therapy than do other forms of DLBCL in this age group. However, *IRF4* rearrangement can occur as a secondary event in other aggressive B-cell lymphomas, and by itself does not define a single entity.

Another follicle center–derived neoplasm presents in the skin, most often involving the head and neck or upper trunk. Primary cutaneous follicle center lymphoma is negative for *BCL2* rearrangement and negative for CD10 but has some of the genetic alterations observed in pediatric-type follicular lymphoma, including 1p36 deletions and mutations in *TNFRSF14*. However, it is negative for most of the mutations seen in classic nodal FL. Similar lesions may present in cutaneous and mucosal sites in the lower female genital tract and share similar biology and clinical features. Primary cutaneous follicle center lymphoma almost never
disseminates beyond the skin and is managed with local therapeutic options in most cases. However, lesions with strong expression of BCL2 protein may represent secondary spread from nodal FL and may require further studies for accurate diagnosis.

**Diffuse Large B-Cell Lymphoma**

Historically, DLBCL was separated on the basis of the cytologic appearance in routine hematoxylin and eosin–stained sections. The two most common variants were centroblastic and immunoblastic. We now recognize that DLBCLs are a complex group of aggressive B-cell lymphomas, with genomic studies illuminating diverse biology that impacts clinical behavior and treatment. The contemporary understanding of DLBCL began >20 years ago with gene expression profiling (GEP) studies that identified two major categories of DLBCL, based on the putative cell of origin. One group resembles germinal center B cells, and the other, activated B cells, showing evidence of a post-germinal center stage of differentiation.46 Clinical trial data provided evidence for the clinical significance of cell of origin with conventional chemotherapy,47 and a potential role for more targeted therapy.48

GEP also led to the recognition of other clinically significant forms of aggressive B-cell lymphoma, such as primary mediastinal large B-cell lymphoma,49,50 leading to improved therapeutic options.51 GEP data also provided biological support for the phenomenon of mediastinal gray zone lymphoma, which forms a missing link between primary mediastinal large B-cell lymphoma and nodular sclerosis classic Hodgkin lymphoma.52 These insights were key to accurate diagnosis of these once perplexing lesions, and have also led to new therapeutic approaches.53–55

Aggressive B-cell lymphomas constitute >15 separate entities.29 Many factors play a role in their subclassification, including viral pathogenesis (eg, Epstein-Barr virus and human herpesvirus 8), or specific genetic alterations (ALK, MYC, BCL2, and BCL6). Clinically, a major emphasis has been placed on so-called double-hit lymphomas, which have translocations of both MYC and BCL2, and can occur de novo, as well as progression from FL. However, more recently, GEP has led to the identification of a double-hit signature that shares features with the dark zone of the germinal center. More importantly, this dark zone signature captures cases that lack MYC and BCL2 rearrangements as observed by fluorescence in situ hybridization, but share similar biology and clinical outcome.56 Thus, this GEP signature will be key to identifying such cases in routine clinical practice.

A comprehensive overview of all the aggressive B-cell lymphomas is beyond the scope of this review. Most recently, the field has focused on the genetic diversity of DLBCL using high-throughput sequencing approaches. Several major studies have identified five to seven distinct genetic subgroups57–60 (Table 1). Most of these genetic subgroups show some enrichment in individual categories of DLBCL identified by their cell of origin (germinal center B cells or activated B cells), but a direct translation of the genetic categories to cell of origin has not been possible. Some of these clusters have major clinical significance and are more immediately applicable to clinical practice. For

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<th>Table 1</th>
<th>High-Throughput Sequencing Studies of DLBCL</th>
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<td>Major hallmarks</td>
<td>Wright et al (2020)56</td>
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<td>Mainly ABC DLBCL</td>
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<td>MYD88265P mutation</td>
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<td>Mainly GCB DLBCL</td>
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<td>EZH2 mutation</td>
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<td>TET2 mutation</td>
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<tr>
<td>Other</td>
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*Approximate percentages are based on the study by Wright et al.56

ABC, activated B cell; C, cluster; DLBCL, diffuse large B-cell lymphoma; GCB, germinal center B cell; NA, not available.
example, the A53/cluster 2 is associated with aneuploidy and TP53 abnormalities, with poor clinical outcome. Another major subtype is MCD/cluster 5, which is found in most primary central nervous system large B-cell lymphomas, and many other DLBCLs arising in extranodal immune-privileged sites. Nevertheless, the currently published data fail to account for all cases of DLBCL. Next-generation sequencing approaches will comprise the next phase in the classification of lymphomas and will be key to advancing the principles of personalized medicine.

Pathology Provides a Roadmap for Disease Discovery and Treatment

Disease discovery and disease definition using routine and more novel diagnostic tools are critical first steps in elucidating the pathogenesis of lymphomas. Discovery of recurrent genetic alterations has usually followed on the heels of a precise description of the lymphoma entity based on clinical, morphologic, or immunophenotypic grounds. Indeed, most of the entities discussed in this review were recognized first by pathologists in clinical practice. In other words, it starts with the microscope but insights from genetics, epigenetics, and knowledge of the cellular microenvironment lead to refinement of diagnostic criteria, and ultimately appropriate therapy.

Disclosure Statement

None declared.

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