The Search for Meaningful Prognostic Markers in Diffuse Large B-Cell Lymphoma

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The diagnosis and classification of non-Hodgkin lymphoma has undergone substantial change during the last 20 years. We have moved from a purely morphologically driven classification system to one in which immunophenotype and molecular genetic features are now part of the disease definitions. This has permitted us to define new entities such as mantle cell lymphoma, anaplastic large cell lymphoma, and extranodal marginal zone lymphoma. Despite much progress, we still recognize that many disease entities are heterogeneous. For example, several studies have demonstrated the t(14;18) (characteristic of follicular lymphoma [FL]) in a substantial minority of diffuse large B-cell lymphomas (DLBCLs), suggesting that DLBCL encompasses more than one entity. Nonreproducible morphologic criteria have helped to place the Revised European American Classification of Lymphoid Neoplasms (REAL classification) provisional entity of Burkitt-like lymphoma into the melting pot of DLBCL in the upcoming World Health Organization classification. Although B-cell immunoblastic lymphoma was discarded in the REAL classification, some maintain that it should be separated from DLBCL with centroblastic features. Immunophenotyping of DLBCL, even with a limited panel of markers commonly used in lymphoma diagnosis, also reveals multiple patterns.

This heterogeneity also is reflected in the clinical behavior of DLBCL. While 50% to 60% of patients with DLBCL are cured with anthracycline-containing regimens, 40% to 50% of patients are not cured. To assist in the clinical stratification of patients with these lymphomas, the international prognostic index (IPI) was developed and has proven to be a very useful tool for clinicians in predicting outcomes of patient groups based on a limited set of clinical and laboratory variables. Although host factors measured by the IPI must be considered important, I believe that the IPI, at least in part, is a surrogate marker for as yet undefined biologic markers.

As a testament to the importance that investigators have placed on finding prognostic biologic markers in DLBCL, numerous studies have been undertaken to find new markers that can identify patients who will not do well with current multiagent chemotherapy. Implied in this endeavor is the supposition that there are biologic differences between lymphomas that will independently affect the clinical outcome. In the current issue of the Journal, Uherova and colleagues describe a series of 28 patients with de novo DLBCL. Patients with histories of FL and cutaneous lymphomas were excluded from the study. In a univariate analysis, the authors report a shorter overall survival in patients with lymphomas expressing CD10 compared with those whose lymphomas lack this marker. This is one of the first studies to examine CD10 status as a prognostic factor in DLBCL. Others, cited by the authors in their article, also are recent, and survival also is compared by univariate analysis. Studies such as these raise important questions: (1) Are there accepted biologic markers that are independent prognostic factors in DLBCL? (2) How does the present study fit into published literature? (3) Might there be other markers that may prove superior to current markers, and how do we find them?

Are There Accepted Biologic Markers That Are Independent Prognostic Factors in DLBCL?

No single marker has achieved universal acceptance as an independent prognostic marker for DLBCL. Although controversial, perhaps the leading candidate markers (or at least most published) to date for DLBCL include proliferative index, p53 gene or pathway alterations, and bcl-2 protein expression. For each of these, studies have presented evidence for and against them as prognostic markers, but
relatively large series with multivariate analyses suggest that these markers do have importance.

Proliferative index as measured by the Ki-67 fraction was shown in 2 studies to be an independent factor for survival.\(^{15,16}\) The study of Miller and colleagues\(^{16}\) determined a cutoff of 80% or more as a poor prognostic indicator, independent of clinical features included in the IPI. These studies were done using frozen section immunohistologic examination. Today, reproducibility in fixed tissues with other antibody clones would need to be addressed. Newer, automated staining systems with online, controlled antigen retrieval protocols might allow reproducible results between laboratories and is a first step toward standardization.

It is difficult to come to a conclusion about the significance of p53 alterations in DLBCL because of the many ways of assessing p53 status, including immunostaining, loss of heterozygosity analysis, single-strand conformational polymorphism analysis, and direct sequencing. Certainly for pathologists, immunostaining represents the simplest means of studying p53. However, it is now well known that p53 overexpression can occur in the absence of mutations.\(^ {20}\) In the study by Maestro and colleagues,\(^ {21}\) many cases of DLBCL with p53 immunostaining lacked mutations. Nevertheless, Piris et al\(^ {17}\) and Zhang et al\(^ {12}\) found p53 immunoreactivity to be an independent factor in survival using multivariate analysis. In another large series, however, Kramer and colleagues\(^ {12}\) did not find p53 to be an independent factor when IPI was considered. One study of Working Formulation intermediate- and high-grade lymphomas (excluding small noncleaved and lymphoblastic lymphomas) did find a much shorter overall survival for patients with p53 mutation in the low/low-intermediate IPI group.\(^ {23}\) To my knowledge, no large series of DLBCL with p53 gene sequencing for mutation detection using multivariate analysis has been published. In a conceptual step forward, Gronbaek and colleagues\(^ {19}\) recently proposed a pathway approach, suggesting that combined p16 and ARF-p53 pathway defects predict poor outcome independent of the IPI in lymphomas with aggressive histologic features. These approaches may prove more useful than analysis of single variables.

Expression of bcl-2 protein is also controversial as a prognostic indicator in DLBCL. However, most recent evidence strongly supports it as a significant biologic marker. Differences might be ascribed to different techniques and criteria for positivity. Early studies reported that bcl-2 protein expression was not a significant prognostic factor.\(^ {17,24}\) Subsequently, others demonstrated significance in disease-free survival\(^ {12,25}\) or disease-free survival and overall survival.\(^ {14}\) In particular, Gascoyne and colleagues\(^ {14}\) found that bcl-2 expression in lymphomas with diffuse aggressive histologic features predicted both for disease-free survival and overall survival, independent of the IPI clinical factors.

How Does the Present Study Fit Into Published Literature?

To date, little has been written about the prognostic significance of CD10 expression in DLBCL. This may change, as paraffin-reactive anti-CD10 antibodies have recently become available. Approximately 29% of the cases were CD10+, similar to the literature reports of approximately 20% to 35%.\(^ {7,26}\) CD10 is expressed in the majority of FLs, although clearly CD10 expression is not specific for it. Nevertheless, Fang and colleagues\(^ {26}\) found that CD10 expression correlated with, and thus may be a surrogate for, the presence of the t(14;18)(q32;q21) in DLBCL. An obvious question is whether many of these CD10+ DLBCLs represent a diffuse high-grade component of undetected FL or a de novo DLBCL with a genetic (and presumably biologic) relationship to FL. Of note, the frequency of CD10 positivity is similar to the frequency of detection of the bcl-2 gene rearrangement in de novo DLBCL.\(^ {1}\) Jacobson et al\(^ {1}\) suggest that presence of the bcl-2 gene rearrangement identifies a group of patients with frequent relapse. Such a course would be similar to that of FL. Others have not found this to be the case and found no difference in relapse or overall survival compared with t(14;18)-negative cases.\(^ {14,18}\) Uherova and colleagues\(^ {9}\) did not study their cases for evidence of a t(14;18).

With regard to prognosis of CD10+ DLBCL, Uherova et al\(^ {9}\) cite similarities and differences with previous studies. Harada and colleagues\(^ {7}\) found that CD5 expression was a significant poor prognostic indicator of overall survival, rather than CD10 expression. On the other hand, Xu et al\(^ {10}\) suggested, in abstract form, a worse outcome for patients with CD10+ lymphoma.\(^ {9}\) Additional studies will be required to resolve these conflicting data before CD10 can be widely accepted as a useful biologic marker.

Might There Be Other Markers That Are Superior to Current Markers, and How Do We Find Them?

In a new paradigm for profiling and grouping DLBCL, gene chip expression arrays were used to examine more than 17,000 genes simultaneously in a series of well-characterized cases.\(^ {27}\) Two major groups were identified—the germinal center–like and the activated B-cell–like lymphomas. Of note, CD10 expression is included in the germinal center–like group. The activated B-cell–like group had a significantly worse survival, even when considering patients with a low IPI.\(^ {27}\) Other investigators using different expression arrays also are beginning to report positive results.\(^ {28}\) Large-scale analyses such as these should provide clues about which candidate markers should receive the most
attention. Once the list of candidates is narrowed sufficiently, prospective studies could be done that focus on expression of a limited number of informative genes. Alternatively, one could perhaps do the same by rapid protein analysis with tissue microarrays. In this manner, one could practically screen and target potentially important biologic markers in large numbers of cases from multiple institutions. The ultimate goal is a useful and practical clinical laboratory test. It seems from the study by Uherova et al.\(^9\) that CD10 might be considered on the "short list" for further study.

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References