A more disease-specific approach to develop clinical prognostic factors would include the characteristic clinical properties of MALT lymphoma. Endoscopic ultrasound examination has been shown to be a highly sensitive technique for analyzing the extent of the disease in the gastric wall in gastric MALT lymphoma. Until now, this parameter has been one of the few to be significantly predictive of the response to therapy in *Helicobacter pylori* eradication protocols for low-grade gastric MALT lymphoma. In view of the essentially different dissemination pattern of extranodal MALT lymphomas stage, as defined by the Ann Arbor system, may not fully appreciate the impact of different lymphoma localization. Staging systems, specifically tailored to these characteristics, may prove to be a more valuable prognosfic factor.

Pathological prognostic factors

In the current model of the evolution of MALT lymphoma, a precursor stage of follicular, *H.* pylori-associated gastritis, is thought to evolve into low-grade gastric MALT lymphoma and ultimately to proceed to transformation to high-grade disease (diffuse large B-cell lymphoma) on the basis of the accumulation of genomic alterations. In the early phases of lymphoma development, the proliferation of tumor cells is still at least partly dependent on immunological drive, mediated through the presence of *H. pylori* antigens. Therefore, two important biological transitions that justify different therapeutic approaches should be recognized by pathologists: the transition from low-grade to high-grade disease and the transition from the antigen-dependent to the antigen-independent phase. Morphologically, the transition to high-grade disease (diffuse large B-cell lymphoma} is marked by dominance of neoplastic blasts in large sheets which are possibly also diffusely intermingled in the infiltrate. These features can be recognized in endoscopic biopsy samples and should be reported to guide the choice of fherapy.

The transition from the antigen-dependent to the antigen-independent phase is much more difficult to recognize. Tumors that have progressed to the antigen-independent phase do not require antigen-mediated T-cell help to support the growth of malignant B-cells. Therefore, removal of the source of antigen in the form of bacterial eradication would not be enough to abolish tumor growth and reach remission in these cases. In a small series of patients (n=22), an increased number of neoplastic blasts (up to 10%) without overt sheets of neoplastic blasts, predicted a worse tumor response to therapy in a *H.* pylori eradication protocol. This suggests that this transition may indeed be morphologically defined.

Immunological and molecular prognostic factors

Many of the known prognostic factors in lymphoma are developed on a trial-and-error basis in large series of patients. Identification of the specific factors that are involved in the development and progression of specific lymphoma entities, however, may help to mark clinically relevant transitions in behavior and may prove to be more essential as prognostic markers. Since the different developmental stages from precursor stages to low-grade and into transformed phases can be recognized, MALT lymphoma is a highly suitable model for identifying these factors. Thus far, p53, *bcl-6, c-myc* and *DCC* have been implicated in the transition from low-grade to transformed disease. Recently, it has been suggested that bcl-10 may be involved in the transition into the antigen independent phase or that it may play a role in transformation. Further research will be needed to dissect the different factors that are involved in clinically relevant transitions. This knowledge may form the basis of the development of specifically tailored treatment protocols.

References

- Hermans J, Krol AD, van Groningen K et al. *International Prognostic Index for aggressive non-Hodgkin's lymphoma is valid for all malignancy grades.* Blood 1995; 86: 1460-1463.
- Krol AD, Hermans J, Kramer et al. *Gastric lymphomas compared with lymph node lymphomas in a population-based registry differ in stage distribution and dissemination patterns but not in patient* survival. Cancer 1997; 79: 390-397.
- Sackman M, Morgner A, Rudolph B et al. *Regression of primary gastdc low-grade MALT lymphoma after cure of Helicobacter pylod infection is predicted by endosonographic staging.* Gastroenterol 1997; 113: 1087-1090.
- De Jong 0, Boot H, van Heerde P *et* al. *Histological grading in gastric lymphoma: Pre-treatment criteda and clinical relevance.* Gastroenterology 1997; 112: 466-1474.
- Ott G, Katzenberger T, Greiner A et al. *The t(11;18)(q21;q21) chromosome translocation is a frequent and specific aberration in low-grade but not in high-grade malignant non-Hodgkin's lymphomas of* the mucosa-associated *lymphoid tissue (MALT-)type. can* Res 1997; 57: 3944-3948.
- Willis TG, Jadayel DM, Du MG et al. *BclIO is involved in t(1;14)(p22;q32) of MALT B cell lymphoma and mutated in multiple tumor types. cell* 1999; 96: 35-46.

Prognostic factors in diffuse large B-cell lymphoma

R. Gascoyne

British Columbia Cancer Agency, Vancouver, Canada.

Introduction

The late 1980s and 1990s have seen a significant increase in our understanding of the immune system and the biology of the non-Hodgkin's lymphomas (NHL). The diversity of the diffuse large B-cell lymphomas (DLBCL) is more readily appreciated with the acceptance of the revised American European lymphoma (REAL) classification and the soon to be released World Health Organization (WHO) classification. A modified version of the diffuse aggressive B-lineage lymphomas, to include expected provisions to the WHO scheme, is shown in Table 1. Modern classification of NHLs is based on a synthesis of morphology, immunophenotypic findings, molecular genetic studies and cytogenetic features. In difficult cases ancillary studies may be required but at a minimum, the determination of lineage is mandatory. Clinically equivalent cases of peripheral T-cell lymphoma have a much worse prognosis than do DLBCLs.

Table 1. Diffuse large 8-cell lymphomas.

Diffuse large B-cell lymphoma subtypes:
Mediastinal (thymic) large B-cell lymphoma
Intravascular large B-cell lymphoma
Primary effusion lymphoma
Diffuse large B-cell lymphoma variants:
Centroblastic
Immunoblastic
T-cell rich 8-cell lymphoma (TCRBCL)
Anaplastic large B-cell lymphoma

A number of clinical variables are now well established as prognostic factors useful for predicting outcome in DLBCL. An international prognostic index score (IPI) has been developed that includes age, stage, serum lactate dehydrogenase, number of extranodal sites and performance status, assembled into a score between 0 and 5. Patients with a score of 4-5 do significantly worse than those with an IPI score of 0-1. Thus, the IPI score is a powerful predictor of survival in DLBCL but importantly, these clinical variables are surrogates that fail to define the underlying biologic heterogeneity of the DLBCLs. Pathologic or "biologic" variables have been sought to provide the correlates that underlie the clinical behavior of these lymphomas. The most useful of the pathological prognostic factors are those that contribute to the determination of prognosis beyond the information gleaned from an analysis of the clinical variables alone. Some of the well-characterized independent factors are listed in Table 2, together with their probable mechanism of action. What follows is a brief review of pathologic prognostic factors in DLBCL.

Morphology

DLBCLs can be divided morphologically into two principal subsets, centroblastic (large noncleaved) and immunoblastic lymphoma (IBL). Several difficulties arise with classification, in particular, no definitive diagnostic criteria exist to distinguish centroblastic lymphoma from IBL. In practice, many cases reveal a mixture of both cytologic types. Several studies have shown that there is poor interobserver reproducibility for distinguishing the cytologic subtypes of DLBC. Nonetheless, recent studies suggest that B-cell IBL may have a more aggressive course. Of potential help for resolving this problem, objective criteria that may prove useful for distinguishing centroblastic lymphoma from IBL have been proposed. The postgerminal center cell derivation of IBL has been used to show that immunoblasts have a characteristic immunophenotype based on the differential expression of Bcl-6, syndecan-1 (CD138) and Epstein-Barr virus (EBV)-related protein LMP-1 (latent membrane protein). IBL is typically Bcl-6 -/CD138+/LMP-1 + in contrast to centroblasts which are Bcl-6+/CD138–/LMP-1 –. These immunophenotypic criteria are deserving of study in retrospective and prospective clinical trials.

T-cell rich B-cell lymphoma (TCRBCL) is a recognized variant of DLBCL. Recent observations suggest morphologic and phenotypical overlap with histiocyte-rich large B-cell lymphoma and a proposed common histogenesis with nodular lymphocyte-predominant Hodgkin's disease. The poor prognosis of TCRBCL appears to be related to its propensity to involve the bone marrow, a well-established adverse prognostic factor. Bone marrow positivity is detected in as many as 50-60% of cases.

Discordant small cell lymphoma may be found in the bone marrow at the time of staging in approximately 5% of *de novo* DLBCL. Interestingly, this finding does not appear to have any impact upon prognosis. However, large B-cell lymphoma involving the marrow at diagnosis is associated with a worse prognosis, as is peripheral blood involvement.

Finally, mediastinal large B-cell lymphoma with sclerosis is included in the WHO classification as a recognized subtype of DLBCL. It is thought to arise from a normal B-cell resident in the thymus, Histological findings include a background of fine sclerosis and a tendency for the neoplastic large B-cells to have moderate amounts of clear cytoplasm. Clinically, this lymphoma shows a female predominance, relatively young age, high serum lactate dehydrogenase, bulky disease associated with superior vena cava syndrome and extension to intrathoracic, extranodal sites. Although still controversial, the majority of published reports suggest a favorable outcome compared with other DLBCLs.

Bcl-2 expression and rearrangement

The *bcl-2* gene was discovered by virtue of its involvement in the reciprocal translocation, t(14;1 8), that characterizes the majority of cases of follicular lymphoma. This leads to the constitutive overexpression of normal Bcl-2, a protein found in a variety of tissues and cells throughout the body. Importantly, Bcl-2 protein is expressed in many lymphoproliferative disorders lacking a t(14;18) *(i.e.,* bcl-2 gene rearrangement), in addition to many other cancers. The t(14;1 8) and its corresponding *bcl-2* gene rearrangement can be found in 15-25% of *de novo* DLBCL cases. This is in contrast to Bcl-2 protein expression which is seen in approximately 25-50% of DLBCL, suggesting that mechanisms other than translocation can upregulate Bcl-2 expression in these lymphomas. Recently, gene amplification has been demonstrated as an alternate mechanism, which results in Bcl-2 overexpression in DLBCL and which appears to occur in cases specifically lacking evidence of a bcl-2 gene rearrangement.

The Bcl-2 protein is antiapoptotic and may interfere with a final, common cell death pathway, normally induced by either chemotherapy or radiation. Thus, lymphomas that overexpress Bcl-2 protein are likely to be relatively radiation- and drug-resistant and associated with treatment failure. Several recent studies have confirmed that the presence of a t(14;18) is not predictive of either overall or failure-free survival in patients with DLBCL, in contrast to Bcl-2 protein expression, which is an independent predictor of outcome in DLBCL. In several of these reports, some cases with a *bcl-2* gene rearrangement failed to express Bcl-2 protein, presumably explained by mutations of the open reading frame of the translo-

Table 2. Biological prognostic factors in diffuse large 5-cell lymphomas.

Molecular defect	Marker	Biological principle
Apoptosis	Bcl-2 protein	Decreased apoptosis, relative chemoresistance
Cell cycle	p53, p21, p27, p16	Loss of growth control
Proliferative fraction	Ki-67, MIB-1	Increased cell division
Transcription factor	*bcl-6* oncogene	?
Adhesion molecules	CD44, ICAM-1	Gain of mobile phenotype
Cytokines	TNF-receptor, IL-10	Autocrine loop leading to enhanced growth?
Drug resistance	P-glycoprotein (mdr-1)	Gain of drug efflux pump

cated *bcl-2* allele. This finding further underscores the lack of a relationship between the presence of the molecular rearrangement and the production of a functional protein. Several additional points regarding Bcl-2 are worthy of note. Expression of Bcl-2 protein is inversely related to proliferative activity in DLBCL, consistent with the hypothesis that Bcl-2 retards entry into the cell cycle. Firstly, these results are difficult to reconcile with the observation that increased mitotic activity is also associated with inferior outcome in DLBCL (see below). Secondly, extranodal DLBCLs have a much lower frequency of Bcl-2 positivity than equivalent nodal histology, a finding that may have an impact upon prognosis.

Bcl-6 rearrangements

The *bcl-6* gene located on chromosome 3q27 encodes a transcriptional repressor normally expressed by B-cells of the germinal center. As normal B-lymphocytes exit the follicle, there is a commitment to differentiation that is associated with decreased expression of Bcl-6 protein. Therefore, Bcl-6 protein expression is a good marker of the germinal center. The observation that rearrangement of the *bcl-6* gene on chromosome 3q27 was an important prognostic marker in DLBCL was initially reported in 1994. These authors described an association with extranodal disease and freedom from disease progression greater than 80% in a series of patients with DLBCL. However, others have been unable to confirm the prognostic significance of *bcl-6* rearrangement. Furthermore, the authors of the original report have subsequently published findings suggesting an inverse relationship between *bcl-6* gene rearrangement and the presence of additional markers of clonal cytogenetic evolution typically associated with a poor outcome in DLBCL. Thus, the prognostic significance of *bcl-6* gene rearrangements in DLBCL remains unresolved.

Reciprocal rearrangements of 3q27 and the immunoglobulin (Ig) genes (14q32, 2p12, 22q11) are common but half of the *bcl-6* translocations may involve other non-Ig loci. These latter cytogenetic rearrangements are less frequently detected with Southern blot analysis, creating a problem with false negative results. Of equal importance, *bcl-6* gene rearrangements are commonly cryptic (subcytogenetic changes) and can be detected by Southern blot analysis even when the 3q27 breakpoint using routine cytogenetics does not appear to be involved. Thus, studies addressing the prognostic significance of the *bcl-6* oncogene in DLBCL are better served by combining both molecular and cytogenetic data. A similar dichotomy exists for the *bcl-6* gene and Bcl-6 protein as is seen with the *bcl-2* oncogene: there is no clear relationship between the presence of the translocation and expression of the protein. A study to determine the independent prognostic impact of Bcl-6 protein expression in DLBCL has not been reported.

Cell cycle regulation

Mutations of the p53 gene are associated with many solid tumors and a number of lymphoid malignancies. For the indolent B-cell NHLs, p53 mutations are closely correlated with p53 protein expression. Additionally, transformation of follicular lymphoma to DLBCL is clearly linked to mutations in p53 and p16, the latter cell cycle protein which is located on chromosome 9p21 encodes a cyclin-dependent kinase. However, the story for DLBCLs is far less clear. P53 expression is frequently detected in de *novo* DLBCL without evidence of mutation, suggesting upregulation of wild-type p53 protein in these cases. Wild-type p53 is responsible for increasing the expression of another related protein called p21, a downstream effector of p53 function normally involved in cell cycle control through the binding and inhibition of cyclin-dependent kinases. P53 mutations have been shown to predict outcome-in OLBOL but the data for p53 protein expression are much less clear. A surrogate marker that may be of value for detecting p53 mutations is dual labeling for p53/p21. The cases with the phenotype p53+/p21— appear to be the cases with p53 mutations and an associated poor outcome. Other cell cycle regulatory proteins of potential interest include Mdm2, retinoblastoma protein, p16, p27 and cdc25b. Preliminary data suggests that some of these factors may have prognostic significance in DLBCL.

Tumor cell proliferation

There are conflicting reports regarding the effect of tumor cell proliferation on clinical outcome in DLBCL. Several methodologies have been used to assess the proliferation rate but more recent studies have utilized immunohistochemistry with either Ki-67 or the paraffin-equivalent, MIB-1. One study reported that a proliferation rate of >80% was associated with poor survival in previously untreated patients with aggressive NHLs, whereas another study found that patients who achieved a good response to treatment were less likely to relapse if they had a tumor cell proliferation of >80%. These data are in conflict and perhaps importantly, are also discordant with recent data concerning Bcl-2. Two studies have reported that Bcl-2 protein expression in DLBCL tends to correlate with a low proliferative index, a finding that is in agreement with preclinical models. Thus, the proliferation rate and Bcl-2 protein expression data are at odds with each other and would suggest that tumors with a low proliferation rate are less sensitive to chemotherapy than are rapidly proliferating tumors. Well-organized, multiinstitutional studies will be required to answer this question definitively.

Adhesion molecules

Few studies of adhesion molecule expression in DLBCL have been performed but hold promise to be useful predictors of outcome. Specifically, both CD44 and intercellular adhesion molecule-i (ICAM-1) expression have been shown to correlate with disease dissemination and a poor outcome in DLBCL. Further studies of other patient cohorts and additional adhesion molecules will be of interest, as the development of certain primary extranodal NHLs may correlate with the expression of a specific marker profile.

Other factors have been considered as potential prognostic markers in DLBCL including major histocompatibility complex molecule expression, tumor-infiltrating lymphocytes, cytokine production by neoplastic large B-cells and drug resistance markers. However, more studies will be required before they can be established as significant predictors of outcome in DLBCL.

Prognostic factors in mantle cell lymphoma

E. Campo

Dept. of Pathology Hospital Clinic, University of Barcelona, Barcelona, Spain.