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

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Definition and general characteristics

Mantle cell lymphoma (MOL) is a lymphoproliferative disorder, which is derived from a subset of naive pregerminal center cells, characterized by a proliferation of atypical lymphoid cells with a monoclonal B-cell phenotype and coexpression of 0D5 (1, 2). Architecturally, MCL usually shows a diffuse or vaguely nodular lymphoid proliferation effacing the lymph node architecture. Transitional areas between nodular and diffuse patterns are common but, in rare cases, nodularity may be prominent and may lead to a misinterpretation as follicular lymphoma (3). A mantle zone pattern has been recognized in some tumors in which the atypical lymphoid cells expand the mantle cell area surrounding a reactive "naked" germinal center (4, 5). However, some nodules may be solid without evidence of residual germinal centers and may represent the malignant counterpart of primary follicles or, alternatively, a massive infiltration and obliteration of the original germinal center. Two cytological variants have been identified, typical and blastic. Typical cases show a monotonous proliferation of small to intermediately sized lymphoid cells with irregular nuclei and scarce cytoplasm. Occasional cases may show a predominance of small lymphocytes with condensed chromatin and rounded nuclei and only rare cells with irregular or indented nuclei. Proliferative activity in typical cases may vary, but it is usually less than 1-2 mitoses per high power field. Blastic variants include a spectrum of intermediate to large cells with round or irregular nuclei and finely dispersed chromatin. Some cases may have pleomorphic nuclei and a more heterogeneous population of cells. Biastic variants have a higher proliferative activity and a more aggressive clinical evolution (2).

MOL is genetically characterized by 11 qi 3 translocations and bcl-1 rearrangement (6, 7). This alteration leads to a constant overexpression of cyclin D1, which plays an important pathogenetic role, probably deregulating cell cycle control by overcoming the suppressor effect of retinoblastoma protein (Rb) and p27KiP1 (8-11). Detection of cyclin D1 may be used as a highly specific marker of MCL because it is expressed in virtually all of these tumors but only in a few reported cases of aggressive variants of chronic lymphocytic leukemia/small lymphocytic lymphoma and a small percentage of cases of multiple myeloma (8). Aggressive variants have additional genetic alterations including inactivation of p53 and p16INK4a tumor suppressor genes (12-14). Recent cytogenetic studies performed with fluorescence *in situ* hybridization (FISH) and comparative genomic hybridization techniques have demonstrated other genetic alterations in MCL that may also play an important role in the development and progression of these tumors (15, 16).

Clinically, MCL presents in elderly males with advanced disease and frequent extranodal involvement, particularly in the bone marrow, gastrointestinal tract and spleen. The clinical evolution is relatively aggressive with poor response to conventional therapeutic regimens. Complete remission is only obtained in 6-35% of cases although it has reached 50% in some reports (2). The median survival of the patients is 3-4 years in different studies. However, the range is variable with a few patients showing a relatively long survival of 5-10 years, whereas in other cases the evolution is rapidly progressive with a survival shorter than 2 years. Different therapeutic strategies have been proposed as alternatives to classic chemotherapy regimens including myeloablative chemotherapy followed by autologous stem cell transplant, purine analogs and interferon-a as well as monoclonal antibodies in combination with aggressive chemotherapy. Although the results are still debatable, it is important to define the parameters associated with the different biological behavior of the tumors in order to determine the best therapeutic strategies according to the risk of the patients.

Prognostic parameters

Different studies have analyzed the prognostic significance of a number of pathological, genetic, molecular and clinical parameters in MCL.

Pathological parameters

Architectural pattern

Three different architectural patterns have been recognized in MCL: mantle zone, nodular and diffuse. Although identification of these patterns is important in the differential diagnosis of MCL, its prognostic value is controversial, due in part to the lack of clear definitions and the frequent presence of a mixture of these patterns in the same case. A mantle zone pattern has been associated with more frequent localized -disease, a higher proportion of complete remissions and longer survival in some studies (17). However, the number of cases with this particular pattern has been very low in most series, precluding confirmation of these results (18-20). Moreover, in older series without detailed molecular or immunophenotypic confirmation, other types of B-cell lymphoma such as MALT lymphomas, may have been included. No clear differences in survival have been observed between cases with a nodular or diffuse pattern (19, 20), although a trend favoring improved survival for nodular cases has been seen (21).

Cytological variants

Different studies have shown that blastic morphology is clearly associated with poor prognosis in patients, with a median survival of 16-18 months, significantly shorter than the 50 months in patients with typical morphology (18, 20). These patients also have a poor response to therapy and usually fail to obtain complete remission. Blastic morphology is associated with other parameters associated with poor prognosis such as high proliferative activity (18), increased cytogenetic alterations (16) and molecular alterations in tumor suppressor genes (12, 13, 22).

Proliferative activity

Several studies have shown that an increased mitotic index is an important prognostic parameter. The exact mitotic index for which prognostic significance may be shown varies, but generally, a mitotic rate higher than 1.5-2.5 mitoses per high power field indicates a more aggressive course (18, 20). Differences between studies may be due to methodological aspects. Similarly, a high proliferative index, recognized by Ki-67/MIB-1 immunostaining, has also been associated with a poor prognosis (21, 23). In fact, mitotic index, S-phase detected by flow cytometry, and Ki-67 labeling are significantly associated in MCL (10). Interestingly, in multivariate analysis different prognostic parameters, including blastic morphology, lose their significance whereas mitotic index remains an independent prognostic factor, indicating that it may be one of the most important predicting factors in these tumors (18). It will be necessary to determine the more appropriate and reproducible method to assess proliferation in these tumors.

Genetic parameters

Classic cytogenic studies have identified additional chromosome abnormalities besides the 11q13 translocations in MCL. Tetraploid clones and aberrant karyotypes have been associated with blastic variants (24). However, these studies are limited and no correlation with the biological behavior of the tumors has been described. Two

recent studies using FISH (15) and comparative genomic hybridization (16) have indicated that the degree of karyotype complexity and the number of chromosomal gains in particular were associated with shorter survival of the patients. Interestingly, the prognostic significance of the number of chromosomal gains was also found when the survival analysis was restricted to patients with typical morphology. In addition, gains in chromosome 3q, 12q and losses in 9p were significantly associated with a shorter survival of the patients.

Molecular parameters

Different studies have analyzed the molecular alterations of MOL. Cyclin D1 expression levels are variable in different cases (8). Cyclin D1 plays an important role in the control of Gi phase by binding to CDK4 and 6. These complexes inactivate the suppressor effect of retinoblastoma on the cell cycle progression. However, the relationship between cyclin D1 expression levels and the proliferative activity of the tumors is not clear, suggesting that other mechanisms may be involved in the proliferative activity of MCL. p53 gene mutations and p16 inactivation have been associated with shortened survival of the patients but these alterations are also closely related to both blastic morphology and a high proliferative rate (12, 13, 25). Interestingly, alterations of these genes have been detected in occasional cases with typical morphology that showed a high proliferative activity and a short survival, similar to cases with blastic morphology (14, 25).

Clinical parameters

The main clinical parameters associated with poor prognosis in published series are advanced age (>65-70 years), poor performance status, advanced stage, splenomegaly, peripheral blood involvement, high lactate dehydrogenase and low albumin serum levels, bulky disease and anemia (2, 18, 20, 21, 23). The International Prognostic Index has been found to be of prognostic value in some series (26, 27) but not in others (18, 28), probably because most of the patients fall into high-risk categories. Muitivariate analysis including clinical and pathological parameters seem to indicate that proliferative activity and performance status of the patients may be independent prognostic factors.

In conclusion, MCL is an aggressive lymphoproliferative disorder with poor response to therapy, frequent relapses, and a short median survival. However, the range of survival may vary from less than 18 months to more than 50 months and a small group of patients may even show a relatively long survival. The parameters associated with this clinical evolution are not well known. Different studies suggest that blastic morphology, proliferative activity, genetic alterations in tumor suppressor genes and different genetic alterations are associated with the prognosis of the patients. The relative impact and independence of these parameters are not clear. Further cooperative studies including larger series of patients are needed for a better understanding of the mechanisms involved in the biological evolution of these tumors.

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