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PROGNOSTIC VALUE OF BIOLOGICAL VARIABLES IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA. CAN WE IMPROVE UPON CLINICAL PARAMETERS?

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ABSTRACT

Background and Objective. Cellular and molecular features including tumor cell proliferation, immunophenotype, adhesion molecule expression and release, and karyotypic abnormalities have been linked with survival in B-cell chronic lymphocytic leukemia (CLL) patients. Although information provided from these studies makes it possible to better appreciate the biological heterogeneity of the disease, it is not clear whether they may substitute clinical features in the prognostic assessment of CLL patients. The objective of this article is to analyze the performance of new prognostic variables, keeping in mind that clinicians should give priority to less expensive biological assays.

Information Sources. In the present review, we examined personal papers in this field, and articles or abstracts published in journals covered by the Science Citation Index and Medline.

State of Art. Clinico-prognostic staging systems do not provide criteria accurate enough to identify patients with progressive disease. Although several *in vitro* methods have been utilized to evaluate tumor cell proliferation and to correlate this parameter with long-term survival, it is difficult to translate the results of kinetic studies into prognosis. Interestingly, prognostic implications of kinetic parameters are exemplified by clinical studies based on lymphocyte doubling time (LDT). Attempts to correlate immunophenotype with prognosis have yielded inconclusive results. This is probably due to the absence of strict immunological criteria. The membrane instability of CD23, rapidly cleaved into a soluble form, provides a highly specific and reliable serum marker. Expression of myelomonocytic antigens (i.e., CD11b, CD13) appears to be restricted to patients with $CD5^-$ CLL. Both cellular expression and release in the serum of some adhesion molecules (i.e., CD54, CD44 standard) have been linked with prognosis. The increased use of fluorescence *in situ* hybridization (FISH) techniques, which make it possible to identify karyotypic abnormalities even in cases with inadequate mytoses, has contributed to a better definition of incidence and clinical relevance of karyotypic abnormalities in CLL. Trisomy 12 is significantly associated with atypical morphological and/or immunological features, high proliferative activity and poor prognosis; the prognostic effect of 11q deletions is consistent in patients under 55 years of age. Usually, in absence of the rearrangement of the bcl-2 locus, B-CLL cells express bcl-2 gene product, whose intracellular levels do not correlate with clinical stages but do correlate with survival, thereby suggesting a possible independent prognostic value. Finally, p53 tumor suppressor gene has been associated with resistance to therapy with fludarabine.

Perspectives. Even though prognostic assessment of CLL patients is generally based on clinical staging systems new biological parameters which reflect the clinical heterogeneity of the disease are under evaluation. Whether in the future clinicians will substitute biological variables for clinical features is matter of debate. The prognostic value of biological parameters is hampered, in some instances, by the small number of patients presenting these features (i.e., 11q deletions in 20%, p53 mutations in 15%). More likely, biological parameters might be incorporated into clinico-prognostic models thus leading to the formulation of a clinicalbiological system for CLL.

Key words: B-CLL, biological parameters, prognosis

Prognostic assessment of B-cell chronic lymphocytic leukemia (CLL) patients is generally based on either Binet or Rai clinical staging.[1,2] These schemes, which reflect the spreading pattern from nodal sites to bone marrow, have some limitations:

1. the criteria used to define clinical stages do not necessarily parallel tumor mass, as mechanisms underlying cytopenia are not considered separately;

2. the criteria are not accurate enough to identify patients with progressive disease.[3,4] Additional clinical parameters have been incorporated into predictive models for CLL, however, they are surrogate variables for the biological heterogeneity of the disease.[5-7] For example, biological features associated with having an advanced clinical stage, rather than

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an advanced clinical stage itself, adversely affect the patient's outcome.

In recent years, cellular and molecular features including tumor cell proliferation, immunophenotype, adhesion molecule expression and release, and karyotypic abnormalities have been linked with survival.[3-7] It is not clear whether the newly identified prognostic parameters will eventually replace, or more likely, integrate clinical variables, thus forming the basis for unique approaches of therapy in specific subsets of patients.

Tumor cell proliferation

Several *in vitro* methods have been utilized to evaluate tumor cell proliferation and to correlate this parameter with long-term survival. The expression of proliferating cell nuclear antigen (PCNA), a 36 kd nuclear protein whose regulation is cell cycle-dependent, has been correlated with lymphocyte doubling time (LDT).[8] According to a recently published study, it would appear that PCNA overexpression may also reflect the intrinsic DNA-repairing activity of leukemic cells, and, consequently, their resistance to chemotherapy.[9]

The proliferative activity of CLL lymphocytes was also assessed in immunocytochemistry by the monoclonal antiboby Ki67. The combination of immunocytochemistry and fluorescence *in situ* hybridization (FISH) made it possible to demonstrate that most Ki67-positive cells were trisomic for chromosome 12, thus suggesting an association between a higher proliferative activity and trisomy 12.[10,11] Despite the interest in *in vitro* kinetic studies, it is not easy to translate the results of biological experiments into prognosis. Interestingly, prognostic implications of kinetic parameters in CLL are exemplified by clinical studies based on LDT.[12-14] Furthermore, integration of the parameters of tumor burden and kinetics allow for the identification of a subgroup of patients belonging to Binet stage A whose survival was not different from that of age-matched healthy controls. For such patients, the term of smoldering CLL was proposed.[15]

Immunophenotypic characteristics and serological disease-specific immunomarkers

Attempts to correlate the immunophenotype with prognosis in B-CLL have yielded inconclusive results.[16-19] This is the case of three large series reporting controversial results on the prognostic role of CD23 expression.[17-19] It is likely that the lack of strict immunological criteria may, at least in part, account for these results that are apparently difficult to interpret. When dealing with B-cell chronic lymphoproliferative disorders, the immunophenotypic diagnosis should be as accurate as possible. To this respect, a combination of five membrane markers makes it possible to distinguish, with a high degree of accuracy, CLL from other B-cell malignancies.[20] Interestingly, the monoclonal antibody CD79b, which recognizes the immunoglobulin-associated membrane protein B29, may further improve the diagnostic accuracy of other B-cell markers.[21]

The membrane instability of CD23, which is rapidly cleaved from the cell surface into a stable soluble form, has important clinico-prognostic implications.[22] Soluble CD23 (sCD23) is a highly specific and reliable serum marker which provides information on both overall survival and freedom from progression of patients in early stages.[23,24] However, there is no definitive evidence that sCD23 may replace clinical parameters that add discriminant power to clinical stages (i.e., BM histology, LDT); more likely, it will be incorporated into the Binet staging system, leading to the division of one intermediate risk group (Rai I-II or Binet B) into two subgroups that differ in prognosis.[24]

The presence of myelomonocytic antigens on the surface of B neoplastic cells was frequently associated with a diffuse pattern of BM infiltration, a feature affecting prognosis independently of clinical stage.[25-27] According to a recent report, it seems that CD13 and CD11b expression is restricted to patients with $CD5^-$ B-CLL, an immunological variant that is known for its poor prognosis.[28]

Cellular adhesion molecule expression and serum release

The expression of certain cell surface antigen molecules belonging to the Ig superfamily (i.e., CD54), proteoglycan (i.e., CD44) and β-integrin (i.e., CD11c) family, has been associated with prognosis in B-cell CLL.[29] As far as the impact on survival of CD44 is concerned, results available in the literature lead to different conclusions.[30,31] The discrepancy between these studies might be explained by the difference of methods (i.e., immunocytochemistry versus flow cytometry) and the specificity of anti-CD44 monoclonal antibody utilized. More recently, the interest of investigators has been attracted by the soluble form of CD44 standard, which is elevated in approximately half of B-CLL patients and reflects tumor burden, disease progression and treatment response.[32]

Among the adhesion molecules belonging to the superfamily of Ig, intercellular adhesion molecule-1 (ICAM-1/CD54) has been studied the most extensively.[33-36] Despite the lack of constitutive cellular expression of such a molecule on B-leukemic cells, increased levels can be found in the serum, thus providing a reliable assay of prognostic relevance.[37-40]

Conflicting data have been obtained on the clinical impact of the expression of CD11c, a β_2 integrin molecule which was reported to be expressed on a wide range, from 13% to 78%.[41-46] As suggested by some investigators, CD11c-positive B-CLL represents a less aggressive form with a better outcome.[18,31]

β_2-microglobulin

β_2-microglobulin (β_2M) is an extracellular protein that is noncovalently associated with α-chain of the class I major histocompatibility complex (MHC) gene which is also detectable in the serum. In either aggressive or indolent non-Hodgkin's lymphomas (NHLs), elevated serum levels of β_2-microglobulin were associated with high tumor burdens and shortened survivals.[47,48] When retrospectively applied to the MD Anderson Cancer Center series of B-CLL patients, β_2M was shown to affect overall survival.[49] Finally, serum β_2M and LDH were two strong independent prognostic variables, and their combination clearly determined three prognostic groups.[50]

Despite the interest in β_2M, to date, there are only two groups that have published in abstract form studies about the prognostic relevance of this marker.[49,50] Furthermore, cutoff is a complex issue with biological markers (i.e., β_2M, sCD23) because we are dealing with a continuous phenomenon.

Karyotypic abnormalities

In B-CLL, clonal chromosome aberrations are detected in approximately 35% of cases by conventional chromosome banding analysis.[51] The most common aberrations diagnosed include trisomy 12, structural abnormalities of chromosome bands 13q14 and 14q32, and deletion of the long arm of chromosome 11 (11q).[51] Chromosome abnormalities are more frequent in advanced phases of the disease, and can be considered acquired events in most instances.[52,53] A significant association between trisomy 12 and CLL with atypical morphologic and/or immunologic features, high proliferative activity, advanced disease and poor prognosis have been reported in several studies, especially in those that employed FISH. Such a technique makes it possible to identify this abnormality even in cases with inadequate mytoses.[11, 54-56] The frequency and clinical impact of 11q deletions in B-CLL by interphase cytogenetics using FISH have been recently analyzed in a consistent patient series.[57] Forty-three out of 214 (20%) patients exhibited 11q deletions, which affected the prognosis of patients under 55 years of age.[57] Given the conflicting data concerning clinical risk factors in younger patients suffering from CLL, this biological finding may be of great relevance in selecting patients with aggressive disease to be considered for intensive treatment approaches.[58-60]

Oncogenes

More than ten oncogenes are implicated as causing CLL, and none of them consistently.[61] Studies reporting the involvement of bcl-1 and bcl-2 genes in 5%-15% of patients have not been confirmed, and involvement of bcl-3 was occasional.[62,63] In the absence of rearrangement of bcl-2 locus, B-CLL cells usually express bcl-2 gene product.[64,65] Although the reason for this is unclear, some preliminary data suggest hypomethylation as a possible cause.[66] Interestingly, bcl-2 overexpression did not correlate with the clinical stage, but did correlate with survival, suggesting a possible independent prognostic value.[67] Bcl-2 belongs to a family of genes whose other known members are Bcl-x_L, Bcl-x_S, and Bax, and either act synergistically with bcl-2 or counteract its activity.[68] An increased bcl-2/bax ratio was particularly pronounced in those patients found to be clinically unresponsive to chemotherapy.[69] Additional genetic abnormalities, including homozygous deletion of a genomic region telomeric to the retinoblastoma (Rb1) gene, overexpression of *c-myc*, and mutation of the p53 tumor suppressor, have been identified in subsets of CLL patients with aggressive disease.[70] As far as p53 mutation is concerned, it is clear that it induces drug resistance and more interestingly, it shortens the survival of mutated patients.[71,72]

Issues in the development of biological prognostic factors

The patients' expectations of receiving an accurate diagnostic and prognostic evaluation have

Table 1. Biological variables associated with clinical features and/or survival.

	Clinical features	*Biological features*
Associated clinical and biological features	Clinical stages BM histology LDT Atypical morphology	β2M, sCD23, sCD54, sCD44, trisomy 12, 11q deletions expression My antigen sCD23, sCD54, PCNA trisomy 12
Prognostically independent biological features		sCD23 β2M
Additional features		p53 mutations 11q deletions bcl-2, bcl-2/bax

PCNA: Proliferating cell nuclear antigen; LDT: Lymphocyte doubling time; BM: Bone marrow.

increased noticeably. Therefore, assessment of prognosis, at the time of diagnosis, should be as accurate as possible. As far as the parameters necessary for a complete pretreatment evaluation are concerned, they differ consistently depending on whether the patient is being treated in the setting of clinical practice or in protocol research. In the clinical practice the ideal diagnostic protocol would be both efficacious and cost-effective.[73] Efforts in identifying predictive prognostic variables should also be addressed in selecting which patient to treat: indeed, such a choice is still complicated.[74]

On the other hand, it is not clear whether prognostic information can be translated into a policy of early therapy, which at the moment can only delay the time of disease-progression without affecting overall survival.[75] Furthermore, as the above-mentioned biological features are evaluated in a larger number of CLL patients, the biological heterogeneity of the disease can be better appreciated. Certain biological features have been correlated with well-known clinical variables, others have been shown to have independent prognostic significance (Table 1). Whether in the future clinicians will substitute biological variables for clinical features is still matter of debate. The prognostic value of biological parameters is hampered, in some instances, by the small number of patients presenting these features (i.e., 11q deletions in 20%, p53 mutations in 15%).[57,71] In the meantime, widely accepted clinical models such as clinical stages, BM histology and LDT should be of aid in the assessment of risk. It is possible, however, that in the future, biological variables may be incorporated into the clinical prognostic models, leading to the formulation of a clinico-biological system for CLL.[76]

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