Adverse Prognostic Features in Chronic Lymphocytic Leukemia

By Sarah Schellhorn Mougalian, MD, Susan O'Brien, MD

The University of Texas MD Anderson Cancer Center, Houston, Texas

ABSTRACT: Patients with chronic lymphocytic leukemia (CLL) can have variable courses, from indolent disease to rapid progression with limited response to treatment. The Rai and Binet staging systems were the first to classify patients into prognostic categories. Newer prognostic markers that correlate with shorter time to progression and time to treatment include elevated serum $\text{M, TK, ZAP-70, and CD38, as well as unmutated IgV}_H$. Abnormal cytogenetics are found in the majority of patients with CLL. Del(17p), as well as mutations of $\text{TP53}$, is associated with an aggressive clinical course and short overall survival. Nearly one-fifth of patients have del(11q) and have a significantly shorter median progression-free survival; mutations in the $\text{ATM}$ gene, located on 11q, may also have adverse prognostic implications. Intermediate-risk cytogenetic findings include trisomy 12 and del(6q). Patients with del(17p) should be evaluated for novel agents and/or allogeneic stem cell transplantation in first remission. Patients with del(11q) require treatment with an alkylating agent in addition to nucleoside analogs and rituximab, and patients with trisomy 12q may express higher levels of CD20, thereby making the malignant cells more susceptible to biologic agents that target CD20. Despite advances in stratifying patients and improved chemoimmunotherapeutic regimens, additional research in prognostication and treatment is needed.

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Chronic lymphocytic leukemia (CLL) is a chronic lymphoproliferative disorder characterized by monoclonal lymphocytosis. Between 2003 and 2008, the age-adjusted incidence rate was 4.2 per 100,000 persons per year, with an annual age-adjusted death rate of 1.5 per 100,000.[1] Although CLL has historically been considered an indolent, slow-growing disease, it progresses rapidly in some patients and they die within 2 to 3 years of diagnosis. Given the heterogeneous nature of the disease course, accurate predictors of prognosis are valuable for the clinical care and counseling of patients. The majority of patients are asymptomatic at the time of diagnosis, and use of a variety of prognostic
measures can predict rapid disease progression and responses to treatment (Table). Although models for determining prognosis for patients with CLL have existed for over 35 years, these relied on simple clinical characteristics such as physical examination findings and blood counts. The complex biologic pathways in CLL are gradually being revealed, and the implications of abnormalities in these pathways, both for treatment and prognosis, are adding to our understanding of this heterogeneous disease.

**Early Prognostic Classification Models**

The first prognostic model was proposed in 1975 by Rai et al. The Rai staging system uses the presence or absence of lymphadenopathy, organomegaly, anemia, and thrombocytopenia to classify patients into one of five stages, stage 0 through stage IV. Median survival for these stages has been found to range from 19 months for stages III and IV to > 150 months for stage 0.[2]

The Binet staging system classifies patients into three categories based on the number of lymphoid tissues involved—including cervical, axillary, and inguinal lymph nodes, as well as liver and spleen—and the presence of anemia and/or thrombocytopenia. Group A patients have no thrombocytopenia or anemia and fewer than three lymphoid tissues involved, group B patients have no thrombocytopenia or anemia but three or more lymphoid tissues involved, and Group C patients have anemia and/or thrombocytopenia. Published median survival rates in these patients ranged from 2 years for patients in stage C to a survival similar to that of age-matched controls for patients in stage A.[3] However, these two staging systems examine patients' clinical status at a single point in time and cannot differentiate between indolent disease and disease that will subsequently become aggressive.

The lymphocyte doubling time, defined as the length of time it takes the absolute lymphocyte count to double from diagnosis, has also been found to have prognostic value in patients with CLL. In one study, those patients whose lymphocyte counts doubled in less than 12 months had a significantly shorter median survival (36 months), whereas those with a longer lymphocyte doubling time experienced longer overall survival (OS) (median survival was not reached).[4] Patterns seen on bone marrow examination also have prognostic value. A diffuse pattern of involvement, including destruction of normal bone marrow architecture and replacement of hematopoietic cells and fat cells with lymphocytes, was associated with decreased OS.[5] Lymphocyte doubling time requires serial blood measurements over time; in addition, the absolute lymphocyte count can vary as a result of other events unrelated to the malignancy. Bone marrow examination is invasive and requires a certain degree of subjectivity, and a diffuse pattern is more often seen in patients who present with already advanced disease. Therefore, these two prognostic factors have significant limitations.

**Newer Prognostic Factors**

Recent research has been directed toward biomarkers and genetic abnormalities that are independent of clinical stage. These abnormalities include IgV<sub>H</sub> mutation status as well as ZAP-70 and CD38 expression.

Serum levels of beta 2 microglobulin (\( \beta_2 \)M), a component of the HLA class I complex on nucleated cells, are elevated in a variety of hematologic malignancies.[6] Measurement of \( \beta_2 \)M in serum is easy, fast, inexpensive, and highly reproducible. Serum \( \beta_2 \)M levels correlate with progression-free survival (PFS).[6-8] Patients with \( \beta_2 \)M levels less than 3.5 mg/L have a substantially longer time to progression than those with levels greater than 3.5 mg/L.[6] and elevated \( \beta_2 \)M levels are associated with lower rates of complete remission and shorter failure-free and overall survival.[9, 10] Adjusting the \( \beta_2 \)M level for creatinine clearance may improve its predictive ability.[11]
Thymidine kinase (TK) is an intracellular protein involved in a salvage pathway for DNA synthesis, so increased expression of TK may reflect increased lymphocyte proliferation. Elevated levels of TK are predictive of disease progression,[6] although there is some disagreement on the optimal value to use to stratify patients. In a small study of 58 patients, TK > 15 U/L was the single best predictor of germ-line IgV_H status, with a predictive value of 86%.[12] Using the value of 8.5 U/L as a cutoff, Matthews et al found that TK level correlated with rapid lymphocyte doubling time, time to progression, ZAP-70 expression, CD38 expression, and poor prognostic cytogenetic findings.[13] TK has been shown to have treatment implications as well; levels greater than 10 U/L are associated with a lower rate of response to treatment with fludarabine.[14]

Mutations in the immunoglobulin heavy chain variable gene (IgV_H), defined as a greater than 2% difference from the germ-line nucleotide sequence, are seen in approximately 50% of patients and are associated with longer survival.[15,16] In one study, patients with IgV_H mutations had a median survival of 293 months, whereas those with the germ-line DNA sequence had a median survival of 95 months.[15] Once patients have been treated, mutation status does not affect the chance of complete remission (CR) in those receiving fludarabine, cyclophosphamide, and rituximab (Rituxan) (FCR); however, patients with unmutated IgVH had a shorter remission duration (47% vs 82% at 6 years).[17] Mutation status remains a prognostic factor even after autologous stem cell transplantation.[18] However, determination of the presence of somatic mutations in the IgVH region requires DNA sequencing, which is not readily available, so the test is difficult to perform in many centers.

Zeta chain associated protein 70 (ZAP-70) is a tyrosine kinase involved in cellular signaling of T cells; it is abnormally expressed in the malignant B cells of some patients with CLL. It is measured by flow cytometry,[19] and its expression has been shown to correlate with the presence of unmutated IgVH genes.[20] ZAP-70 overexpression has been observed in 36% to 57% of study populations;[21-24] however, these trials have used different cutoff values for abnormal expression. It is now generally accepted that expression of ZAP-70 in the cytoplasm of more than 20% of cells represents abnormally high expression.[23,24] ZAP-70 positivity portends a poor prognosis and a shorter time to treatment compared with ZAP-70 negativity; times to treatment were reported as 34 vs 130 months in one study[24] and 2.6 vs 8.4 years in another.[23] The level of ZAP-70 expression was not found to change significantly over the duration of the study.[24] ZAP-70 is highly expressed by T cells, which may cloud interpretation of results; in addition, unlike 2M, it is an intracellular protein, which makes flow cytometry more difficult. Measurement of ZAP-70 by flow cytometry is also highly variable and can depend on the conditions of the sample, its handling and preparation, and the methodology and materials used.[23,25]

CD38, a cell surface glycoprotein normally expressed in a wide variety of hematopoietic cells,[26] is easily measured by flow cytometry in the laboratory.[24] Like ZAP-70, CD38 positivity is associated with faster progression, resistance to treatment, and decreased OS; its expression also correlates with IgVH mutation status.[27,28] In one study, however, there was a 28% discordance rate between CD38 expression and IgVH mutation status, indicating that they are independent prognostic indicators.[27] Studies have reported that 29% to 47% of patients demonstrate CD38 positivity (CD38+), which in most cases (but not all) is defined as expression in 30% or more of cells as determined by flow cytometry.[16,23,24,26-28] Rassenti et al reported a clinically significant difference of 1.7 years between diagnosis and first treatment for CD38+ vs CD38- patients (4.0 vs 5.7 years).[23] Reported median survival for CD38+ patients is shorter (range, 30 months to 10 years) compared with that in CD38- patients (range, 100 months to longer than the study period).[16,24,26-28] CD38 levels may change throughout the course of the disease.[27]
Cytogenetic abnormalities are quite frequent in patients with CLL. Older methods used to perform conventional cytogenetic analysis reported cytogenetic abnormalities in 56% of patients,[29] but these techniques required cell culture and the use of B-cell mitogens to prompt cell division in order to count metaphases. With the advent of fluorescence in situ hybridization (FISH), the ability to detect chromosomal abnormalities is enhanced, since both cells undergoing mitosis and cells in interphase can be examined.[30] In addition, the results obtained using bone marrow and serum specimens are equivalent. Using FISH, Dohner et al found that 82% of patients had abnormal cytogenetics.[31] The cytogenetic profile can change over time; Shanafelt et al examined untreated early-stage patients and found that clonal evolution occurred in 18 of 159 patients (11%); 17 of these changes were found after 5 years of follow up.[32]

FIGURE

Chromosomal Abnormalities and Survival in Chronic Lymphocytic Leukemia

Deletion of the short arm of chromosome 17 (del[17p]) is associated with a very aggressive clinical course[31] and is predictive of decreased progression-free survival (PFS), lack of response to therapy, short response duration, and short OS (Figure).[31,33,34] Del(17p) has been described in as few as 7% of patients naïve to treatment and in as many as 33% of patients with advanced, relapsed disease.[31,35,36] Most patients with del(17p) have other associated chromosomal abnormalities.[37] However, there is some variability in prognosis among patients who carry a 17p deletion,[37] and not all patients with this deletion are destined to have rapidly advancing disease.

Tumor protein 53 (p53 or TP53) is a tumor-suppressor protein encoded by the TP53 gene, which is located on the short arm of chromosome 17.[38] TP53 mutations have been described in 8.5% to 15% of patients, with the majority seen in patients with del(17p).[39-44] However, there is a small subset of patients (3.1% to 4.5% of patients) who have TP53 mutations in the absence of del(17p). [41,43] TP53 mutations are more prevalent in progressive and refractory CLL.[44] A TP53 mutation is an independent predictor of poor prognosis and confers even shorter OS than del(17p) in the absence of a TP53 mutation.[41]

Chromosome 11q is deleted in 18% to 20% of patients with CLL.[31,35,36] Patients with 11q deletions (del[11q]) have more aggressive disease, shorter intervals between diagnosis and first treatment (9 months vs 43 months), more prevalent B symptoms, and more extensive lymphadenopathy.[35] In the same study, in younger patients (< 55 years), those with 11q deletions had significantly shorter median survival (64 months vs 111 months).[35].

Similar to the case of del(17p) and TP53 mutations, mutations of the ataxia telangiectasia mutated (ATM) gene may have prognostic implications independent of those associated with the deletion of chromosome 11q, where it is located. ATM gene mutations have been described in approximately 12% of patients, and compared with ATM/TP53 wild-type, are associated with decreased treatment-free survival (40 months vs 130 months) and OS (85 months vs 217 months).[45] In another study by Austen et al, 36% of patients with the 11q deletion also had ATM mutations in the remaining allele, and these patients had a more aggressive course and were more resistant to traditional chemotherapeutic agents.[46] Germ-line mutations in the ATM gene in patients with CLL have also been described; it has
been suggested that patients with a mutation in the *ATM* gene may be predisposed to the development of CLL.[47]

Another common chromosomal aberration is trisomy 12, which is seen in 13% to 16% of patients.[31,35,36] The OS for these patients has been reported to be approximately the same as that in patients with normal genetics,[31] which means that these deletions should thus be classified as intermediate risk. Deletion of the long arm of chromosome 6 is a rarer cytogenetic abnormality seen in patients with CLL (6% to 7% of patients).[31,48] It is generally considered an intermediate-risk feature, since it is associated with more prominent lymphocytosis with atypical morphology, splenomegaly, higher rates of CD38 positivity, and no association with IgVH mutation status.[48]

Although there are cytogenetic abnormalities that are associated with poor outcomes, several cytogenetic patterns predict a more indolent disease course. Patients with normal cytogenetics, who comprise 18% of all patients, have a median survival of 111 months.[31] A deletion on the long arm of chromosome 13 (del[13q]) is seen as the sole abnormality in 18% to 36% of patients[31,36] and is combined with other cytogenetic abnormalities in 19%.[31] When it is the only cytogenetic abnormality, del(13q) confers a better prognosis; in one study, median survival was 17 years, compared with 13.2 years for patients with normal cytogenetics.[32]

Cases of patients with infrequent cytogenetic abnormalities have also been described. Two cases of patients with a deletion of the long arm of chromosome 5 (5q-) but no other cytogenetic abnormalities have been reported; these patients were still Binet stage A at 18 and 28 months after diagnosis, which suggests that this abnormality portends a more indolent course.[49] Other rare cytogenetic abnormalities have been described, including involvement of chromosome 8,[50,51] but more research is needed to determine their prognostic significance, if any.

**Treatment Implications**

The presence or absence of cytogenetic defects can also be used to guide treatment strategy and to predict response to treatment, distinguishing those patients who will benefit from standard treatment from those who will likely be treatment-refractory.

Patients with 17p deletions are known to be refractory to purine analogs[33] and to have markedly worse outcome with front-line standard-of-care treatment with FCR; the subset of patients with 17p deletions receiving FCR had a PFS of 11.3 months, compared with 51.8 months in the entire cohort receiving FCR.[52] Treatment with alemtuzumab (Campath) is effective in patients with 17p deletions, although they still have shorter survival than patients with better-risk cytogenetics. In a front-line randomized trial comparing alemtuzumab to chlorambucil, patients with del(17p) who received alemtuzumab had a longer PFS (10.7 months vs 2.2 months); in that study, patients with normal cytogenetics had a median PFS of 19.9 months and 14.3 months with alemtuzumab and chlorambucil, respectively.[53] In another study of alemtuzumab, in patients who had received prior treatment, OS in patients with del(17p) was 19.1 months, compared with 27.5 months for patients with normal cytogenetics.[36] Although alemtuzumab may somewhat mitigate the poor prognosis associated with del(17p), the PFS and OS of these patients are still inferior to those in patients with good-risk cytogenetics. These patients are therefore candidates for investigational therapies in the front-line setting, and if otherwise healthy, for stem cell transplant in first
remission. Stem cell transplantation can produce durable PFS and OS in as many as 50% of patients.[54,55]

The 11q deletion also has important implications for treatment. Alkylating agents such as cyclophosphamide have been shown to improve response in patients with the 11q deletion. In US Intergroup Trial E2997, in which patients received fludarabine plus cyclophosphamide (FC) or fludarabine alone (F), patients with del(11q) who received FC had similar PFS to those patients with trisomy 12 or normal cytogenetics who received the same regimen.[56] The addition of rituximab does not abrogate the poor prognosis associated with del(11q); in one study, patients with poor-risk cytogenetics (83% of whom had del[11q]) had significantly inferior PFS and OS.[57] In the ongoing intergroup trial (Cancer and Leukemia Group B [CALGB] 10404), a four-arm study comparing fludarabine and rituxumab (FR) to FCR with and without 4 months of lenalidamide (Revlimid) maintenance, all patients with 11q22 deletions who were originally randomly assigned to treatment with FR are re-assigned to treatment with FCR followed by lenalidamide once FISH results are available.[58] Another study suggests that del(11q) may respond better to immunochemotherapy with FCR; the 11q22 deletion was undetectable in 25 of 27 patients after treatment.[59] Furthermore, treatment with pentostatin (Nipent), cyclophosphamide, and rituximab (PCR) resulted in no difference in PFS between patients with 11q22 deletions and those with other abnormalities (excluding 17p-).[60]

Patients with trisomy 12 have high rates of response with rituximab-containing therapy, possibly because of their higher levels of expression of surface CD20. [61]

Conclusion

Our ability to stratify patients with CLL into high-risk and low-risk categories has advanced dramatically over the past two decades. However, which test or tests are most reliable remains to be seen. Overall, treatment outcomes have improved with the advent of chemoimmunotherapy regimens. Cytogenetic and molecular characterization of neoplastic cells in CLL is becoming increasingly important in predicting clinical course and determining best first-line treatment. Additional research into investigational therapies to target different cytogenetic and molecular profiles is necessary.

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