Pathology Consultation on Evaluating Prognosis in Incidental Monoclonal Lymphocytosis and Chronic Lymphocytic Leukemia

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Abstract

Chronic lymphocytic leukemia (CLL) is a monoclonal B-cell lymphoproliferative disorder generally characterized by an indolent clinical course. However, some patients with CLL will have more aggressive disease progression, and identifying that subgroup may be important for early, or perhaps more aggressive, intervention. In addition, monoclonal B-cell lymphocytosis is often found on routine laboratory evaluation, and it is important to distinguish this entity from overt CLL. Moreover, since many patients with CLL are discovered incidentally and before significant disease progression, prognostic laboratory evaluation may become increasingly efficacious as therapeutic options replace the older strategy of expectant observation. Prognostication may be especially critical if it correctly identifies patients with early stage CLL who are at high risk of clonal evolution and/or resistance to chemoimmunotherapy. Laboratory studies include surface CD38 and intracellular ZAP-70 expression by flow cytometry, serum β₂-microglobulin, and immunoglobulin heavy-chain variable gene mutational status. Cytogenetics for targeted chromosome alterations may similarly aid in predicting outcome and guiding early intervention. This article concisely reviews the utility of commonly performed prognostic markers and addresses the laboratory evaluation in patients with incidentally discovered early stage CLL.

Case Scenario

A 55-year-old healthy man being evaluated for an insurance physical is found to have a WBC count of 17 × 10⁹/L with 55% lymphocytes; the hematocrit and platelet count are within normal limits. Morphologic evaluation of the peripheral smear is remarkable for a homogeneous population of small, mature lymphocytes with condensed and cleaved chromatin; a significant proportion of prolymphocytes is not seen. Flow cytometry evaluation of blood demonstrates that 40% of white cells (6.8 × 10⁹/L) are κ (low-density)–restricted CD19+ B cells, which coexpress CD20 and IgD (both dimly), as well as CD5, CD43, and CD23, but do not express CD10, CD103, CD11c, CD25, CD38, or FMC7. The flow interpretation is “involvement of blood by monoclonal B-cell lymphoproliferative disease consistent with chronic lymphocytic leukemia (CLL).” The patient has no complaints and no physical findings; the β₂-microglobulin is normal, and there is no lymphadenopathy by examination or on imaging of the chest and abdomen. You are consulted by the patient’s internist as to whether additional laboratory testing will be helpful for decision making as to future management.

Questions

1. Does the diagnosis of true CLL vs monoclonal B-cell lymphocytosis with CLL phenotype affect prognostic evaluation?
2. Is there evidence that prognostic laboratory evaluation should be done in patients with CLL at the earliest clinical stages?
3. Which prognostic assays have the best clinical utility?
4. What, if any, prognostic studies should be performed in this patient?

**Defining CLL and Monoclonal B-Cell Lymphocytosis Is Important for Prognosis**

CLL is the most common adult leukemia affecting the Western world, with an incidence of about 4 per 100,000 and a median age at diagnosis between 70 and 80 years (>80% diagnosed after age 60 years). World Health Organization (WHO) criteria define CLL as greater than or equal to $5.0 \times 10^9$ L monoclonal B cells in peripheral blood. The monoclonal mature B lymphocytes show dense, aggregated chromatin, and the typical immunophenotype demonstrates CD19+ CD20dim+ CD5+ CD23+ CD10– B cells with dim surface light-chain restriction. As the elderly population expands and flow cytometric immunophenotyping increasingly provides a very high level of detection, more individuals are incidentally found to have a small population (<$5.0 \times 10^9$/L) of circulating monoclonal B cells with these CLL-specific markers, an entity defined as monoclonal B-cell lymphocytosis (MBL) with a CLL phenotype. MBL represents a premalignant B-cell state similar to monoclonal gammopathy of uncertain significance. About 3% to 4% of the “healthy” population can be shown to have MBL. One analysis has shown that MBL always precedes CLL, with a range of 6 months to 6 years, but it is estimated that only 1% to 2% of patients with MBL will progress to CLL each year. Interestingly, MBL carries the identical frequency of cytogenetic abnormalities (11p–, 13q–, 17p–, and trisomy 12) and immunoglobulin heavy chain variable (IGHV) gene mutations, yet its clinical status as an indolent “premalignant” condition trumps all other prognostic data. It has yet to be elucidated whether a pathogenetic subgroup of MBL will subsequently predict which patients will later develop CLL. Thus, the recommended management of patients with MBL is an annual physical examination and complete blood count; no other laboratory studies or invasive procedures are warranted due to the very low progression rate of MBL to CLL.

**Clinical Staging of CLL Is Most Useful for Patients Beyond the Earliest Stage**

Risk stratification is critical for disease management since the median survival for all patients with CLL is widely variable and, depending on prognostic factors, ranges from 18 months to greater than 10 years. Treatment has traditionally been reserved for patients with active disease that includes anemia or thrombocytopenia, lymphadenopathy, hepatosplenomegaly, progressive lymphocytosis (doubling time of <12 months), or severe constitutional symptoms. In addition, age and performance status (often related) based on clinical evaluation will likely affect whether a patient is eligible for aggressive therapy. Two clinical staging criteria—the Rai system (common in North America) and the Binet system (widely used throughout Europe)—classify patients into 3 relatively parallel groups with distinct prognoses.

**Table I**

<table>
<thead>
<tr>
<th>Stage</th>
<th>Definition</th>
<th>Median Survival, y</th>
</tr>
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<tbody>
<tr>
<td>Rai system</td>
<td>Low risk</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>Lymphocytosis only (&gt;5 x 10^9/L monoclonal B cells)</td>
<td>12</td>
</tr>
<tr>
<td>Intermediate risk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>Lymphocytosis and lymphadenopathy</td>
<td>7</td>
</tr>
<tr>
<td>II</td>
<td>Lymphocytosis with hepatomegaly and/or splenomegaly (± lymphadenopathy)</td>
<td></td>
</tr>
<tr>
<td>High risk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>Lymphocytosis and Hb &lt;11.0 g/dL (± lymphadenopathy/hepatosplenomegaly)</td>
<td>&lt;1</td>
</tr>
<tr>
<td>IV</td>
<td>Lymphocytosis and platelets &lt;100 x 10^9/L (± lymphadenopathy/hepatosplenomegaly)</td>
<td></td>
</tr>
<tr>
<td>Binet system</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>Hb ≥10.0 g/dL, platelets ≥100 x 10^9/L, &lt;3 lymph node regions involved</td>
<td>9</td>
</tr>
<tr>
<td>B</td>
<td>Hb ≥10.0 g/dL, platelets ≥100 x 10^9/L, ≥3 lymph node regions involved</td>
<td>5</td>
</tr>
<tr>
<td>C</td>
<td>Hb &lt;10.0 g/dL or platelets &lt;100 x 10^9/L</td>
<td>2</td>
</tr>
</tbody>
</table>

Hb, hemoglobin.
progress faster to a need for therapy than those with a 0 to 1 risk factor.14 *IGHV* mutational status was a similarly powerful discriminator of progression, and (although not specifically examined) the study did not preclude detection of 17p– at diagnosis as another prognostic marker.14

Therefore, it may be important to compare expectant therapy with a more aggressive, but selective, strategy for early treatment of individuals with CLL with laboratory evidence of poorer outcome. Indeed, as more treatment options become viable for patients with CLL, especially those with low to intermediate risk, more laboratory assays may be clinically validated to gauge individual patient prognosis and guide treatment strategy. Table 2 lists current laboratory analyses that are used to assess CLL prognosis. Some of the listed assays15-20 are still investigational, albeit promising, and will not be further discussed in this review.

**Validity of Prognostic Laboratory Assays in CLL**

Somatic hypermutation in the rearranged *IGHV* genes in CLL cells confers a better prognosis than those that lack hypermutation.21,22 *IGHV* genes encode the antigen-binding domain of the B-cell antigen receptor (surface immunoglobulin). In 1 study, patients with mutated *IGHV* genes (<98% identity to germline) had a median survival of more than 25 years, whereas those who had unmutated genes (≥98% identity) had a median survival of less than 8 years.21,22 *IGHV* mutational status at diagnosis tends to remain constant over the course of disease, and its prognostic value has been confirmed in numerous subsequent studies.

There is some correlation between *IGHV* mutational status and CLL expression of CD38. This transmembrane glycoprotein reflects proliferative status and modulates intracellular signaling. Nearly all patients with CLL with 30% or more monoclonal B cells expressing CD38 have unmutated *IGHV* genes and higher levels of monoclonal lymphocytosis,23,24 and this patient group also has shorter overall survival22-24 and a briefer interval to treatment.25 However, CD38 expression and *IGHV* gene status have been shown to be discordant in up to 30% of patients at CLL diagnosis,23 and in clinical trials, *IGHV* analysis has been held as the more reliable indicator for prognosis.26 Technical challenges rarely arise in assessing *IGHV* status; therefore, although CD38 analysis is often performed at diagnosis, *IGHV* status is the more definitive prognostic marker. Since CD38 has been shown to be dynamic throughout the CLL disease course, possibly representing proliferative activity,27 an upward trend in expression may be useful as a real-time indicator of disease progression.28

The ζ-associated protein, with a molecular weight of 70 kDa (ZAP-70), is another potential surrogate for *IGHV* gene status. Intracellular ZAP-70 expression (≥30% of CLL cells) nearly always demonstrates an unmutated *IGHV* gene29,30 and, like CD38, ZAP-70 expression can be quantitated by routine flow cytometric evaluation. However, ZAP-70 has even poorer concordance with *IGHV* status than CD38.31,32 Moreover, the quality control for quantitation of ZAP-70 is subjectively based on either natural killer/T-cell or normal B-cell populations, which may not be present in particular patients with CLL. ZAP-70 expression has also been noted to have both significant variability between laboratory tests and deterioration of expression over time.33 The latter caveat is especially problematic if the patient sample is sent to a reference laboratory. Hence, it is unlikely that ZAP-70 can replace determination of *IGHV* gene status.

Although atypical lymphocyte morphology and elevated doubling time (<12 months) are cited for predicting outcome, the former is too subjective to be a reliable indicator, and the latter evaluation generally cannot be accomplished at diagnosis. Serum levels of β2-microglobulin and thymidine kinase have been demonstrated to be independent biological predictors of progression-free survival, especially in early stage CLL.14 The former test is readily available and should be part of the prognostic evaluation for CLL if multiple prognostic assays are being evaluated for treatment decisions; however, thymidine kinase is not widely available as a clinically useful as a real-time indicator of disease progression.28

The **Table 2** lists current laboratory analyses that are used to assess CLL prognosis. Some of the listed assays15-20 are still investigational, albeit promising, and will not be further discussed in this review.

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**Table 2**

**Prognostic Laboratory Features in Chronic Lymphocytic Leukemia (CLL)**

<table>
<thead>
<tr>
<th>Good Prognosis</th>
<th>Poor Prognosis</th>
</tr>
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<tbody>
<tr>
<td>Normal β2-microglobulin</td>
<td>High β2-microglobulin</td>
</tr>
<tr>
<td>&lt;30% CLL cells express sCD38a</td>
<td>≥30% CLL cells express sCD38a</td>
</tr>
<tr>
<td>&lt;20% CLL cells express cytoZAP-70b</td>
<td>≥30% CLL cells express cytoZAP-70b</td>
</tr>
<tr>
<td>TP53 present (2 copies)/unmutated Mutated (&lt;98% identity) <em>IGHV</em> gene</td>
<td>TP53 loss/mutation Unmutated (≥98% identity) <em>IGHV</em> gene</td>
</tr>
<tr>
<td>Lymphocyte doubling time &gt;12 mo</td>
<td>Lymphocyte doubling time &lt;12 mo</td>
</tr>
<tr>
<td>Trisomy 12</td>
<td>Deletion of 17p13 or 11q23</td>
</tr>
<tr>
<td>Future laboratory studies needing validation: elevated serum thymidine kinase;15 abnormal serum-free light chains;16,17 increased interleukin 8;18 and decreased microRNA expression19,20</td>
<td></td>
</tr>
</tbody>
</table>

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a Surface CD38 expression.

b Cytoplasmic ZAP-70 expression.
and a normal mutation, lymphocyte doubling time greater than 12 months, a mutated IGHV within any clinical stage of CLL.

Most reference laboratories provide fluorescence in situ hybridization (FISH) panels, which specifically incorporate conserved cytogenetic abnormalities as prognostic variables in CLL, including trisomy 12, and deletions of 11q, 13q, and 17p. Deletion of 13q is the most common finding in CLL and does not convey clear risk changes, but trisomy 12 generally confers a better prognosis. By contrast, 11q– and 17p– are poor prognostic indicators. In particular, deletion of 17p13 always includes the TP53 tumor suppressor gene, and more than 80% of monoallelic 17p– patients with CLL will have a mutation in the remaining TP53 gene. Moreover, multiple studies show that patients with 17p– or definitive TP53 deletion/mutation will have extremely brief progression-free intervals after chemotherapy for CLL and rarely go into remission/mutation will have extremely brief progression-free survival. However, he presents at a much younger age than the median for CLL; therefore, laboratory studies to determine prognosis are recommended, including blood for IGHV gene status and marrow for karyotype and FISH panel. These tests respectively reveal an unmutated (100% germline) IGHV gene and 13q–. After discussion between the patient and his hematologist, the patient is referred to a National Institutes of Health cancer center with active clinical trials for CLL.

Proposed Laboratory Risk Stratification for Incidental/Early Stage CLL

Because of their low risk for progressing to CLL, patients with MBL do not require prognostic marker evaluation and should be expectantly followed. Second-line patients with CLL with an advanced clinical stage (Rai III-IV or Binet B-C) generally require therapy no matter what their prognostic assays show. It is truly the low clinical stage (Rai 0-II, Binet A) patients with CLL who most need evaluation of prognostic markers to place them into high- and low-risk categories. The subgroup at lowest risk for progression has a mutated IGHV status, no 11q deletion or 17q deletion/TP53 mutation, lymphocyte doubling time greater than 12 months, and a normal β2-microglobulin. Low clinical stage patients with CLL who are at high risk for progression will have 1 of the following: unmutated IGHV status, 17q deletion or TP53 deletion/mutation, or the combination of at least 2 other risk factors, including 11q–, high CD38, significant (>13 × 10⁹/L) monoclonal lymphocytosis, and elevated β2-microglobulin. Such patients should be considered for clinical trials of early intervention vs watchful waiting.

Case Summary

Our patient meets the phenotypic and WHO criteria for CLL; he has more than 5 × 10⁹/L monoclonal B lymphocytes and thus does not have MBL. The patient’s low clinical stage (absence of organomegaly and lymphadenopathy), low β2-microglobulin, and lack of CD38 expression place him in a group that would generally be characterized by long-term progression-free survival. However, he presents at a much younger age than the median for CLL; therefore, laboratory studies to determine prognosis are recommended, including blood for IGHV gene status and marrow for karyotype and FISH panel. These tests respectively reveal an unmutated (100% germline) IGHV gene and 13q–. After discussion between the patient and his hematologist, the patient is referred to a National Institutes of Health cancer center with active clinical trials for CLL.

References


