The Plasma Cell Labeling Index: A Valuable Tool in Primary Systemic Amyloidosis

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The plasma cell labeling index (LI) is of value in predicting prognosis in multiple myeloma. Primary systemic amyloidosis (AL) is a plasma cell dyscrasia that shares many features with myeloma. We obtained bromodeoxyuridine LI on 125 patients who presented with AL, 22 of whom also had overt multiple myeloma. Forty-six patients had a plasma cell LI > 0%. Of the 46 patients with an elevated LI, 19 (41%) had multiple myeloma as compared with three (4%) of the 79 patients with an LI = 0 (P < .0001). A response to chemotherapy was seen in 14 (30%) of 46 patients with an LI > 0, as compared with ten (13%) of 79 patients with an LI of 0 (P = .015). The median survival of the high LI group was 14.6 months v 29.8 months for the low LI group (P = .02). In the low LI group, 29% are projected to be alive at 60 months, as compared with 20% in the high LI group. When patients with myeloma were excluded from the analysis, the LI did not predict response but continued to indicate a survival disadvantage (P < .06). The major utility of the LI was in identifying those patients most likely to have multiple myeloma and those AL patients with a poor prognosis (median survival, 14.1 months).

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THE PLASMA CELL labeling index (LI) probes the biology of the plasma cell.1 This test is capable of recognizing plasma cells actively synthesizing DNA and can, therefore, differentiate proliferative from nonproliferative cells.2 The plasma cell LI has been repeatedly shown to be clinically useful in the classification and diagnosis of monoclonal gammopathy of undetermined significance, multiple myeloma and its plasmablastic subset, as well as smoldering multiple myeloma.3,4 The plasma cell LI also provides useful information about prognosis and the need for therapy at different times in the course of the disease.5

Primary amyloidosis (AL) is a plasma cell dyscrasia that shares many features with myeloma.6 Patients with AL typically have a monoclonal protein in their serum or urine and bone marrow plasmacytosis. Sometimes, diagnostic confusion arises when the two coexist in the same patient. Because AL is an uncommon disorder, no study of the basic kinetics of the plasma cell has been undertaken. To learn about the plasma cell kinetics and to assess clinical utility, we studied the LI in patients with AL. We wished to address whether the LI had any value in predicting response or survival in them.

MATERIALS AND METHODS

Study subjects. One hundred twenty-five patients with AL seen at the Mayo Clinic between January 1985 and June 1987 were studied. All patients had histologic proof of AL and results from posterior iliac crest bone marrow aspiration and biopsy. No patients with localized, familial, senile, or secondary amyloidosis were included. Approval was obtained from the Institutional Review Board for these studies. Patients were informed that bone marrow samples were obtained for research purposes and that their privacy would be protected.

The patients were divided into two groups: those with multiple myeloma and those without.7 One fourth of patients with AL without myeloma have >10% plasma cells in the bone marrow.5 Patients with bone marrow containing 10% to 20% mature plasma cells and an M protein <1 g/dL were classified as having AL without myeloma, if they had normal calcium and creatinine levels and normal bone radiographs. These criteria are more stringent than those of the Chronic Leukemia-Myeloma Task Force. Patients were classified as responders or nonresponders to therapy based on standard criteria for myeloma.3 Response criteria also required 50% reduction in albuminuria without an increase in serum creatinine or normalization of hepatomegaly measured below the right costal margin in conjunction with normalization of the increased alkaline phosphatase level. Group comparisons were made by chi2 analysis. Survival differences were measured by log-rank and Gehan-Wilcoxon analysis.

Labeling index technique. Bone marrow mononuclear cells were isolated from a Ficoll-Hypaque preparation, and 106 cells were incubated with 10 μmol/L bromodeoxyuridine10 and 1.0 μmol/L fluorodeoxyuridine. Cytocentrifuge slides were prepared, air dried, and fixed in 95% ethyl alcohol. Mouse antibrmodeoxyuridine (BU-1) (20 μg) was placed on the slides for 30 minutes followed by a wash with phosphate-buffered saline.11 Fluorescence was achieved by using rhodamine isothiocyanate (RITC)-labeled goat antiamuine IgG (8 μg). Plasma cells were also identified by immunofluorescence microscopy. Fluorescein isothiocyanate (FITC)-labeled antihuman κ or antihuman λ reagent was added and incubated at 37°C for an additional 30 minutes.12 Labeled cells were identified by bright orange-red immunofluorescence localized exclusively to the nucleus, indicating the incorporation of bromodeoxyuridine into nuclear DNA. This labeling was associated with simultaneous bright green immunofluorescence localized to the cytoplasm, indicating the presence of cytoplasmic immunoglobulin light chain.13 The LI was calculated as the percentage of 500 cytoplasmic immunoglobulin-positive cells showing nuclear fluorescence.

RESULTS

Of 125 patients with AL studied, 22 (18%) fit the criteria for multiple myeloma (Fig 1). The LI was a significant predictor of associated myeloma in AL. Nineteen (41%) of 46 patients with LI > 0% had myeloma (3%) of 79 patients with LI = 0% (P < .0001; x2 = 28.2). As Fig 1 illustrates, the distribution of LI is typical for an unselected group of myeloma patients.13 Increased LI helped predict which patients would respond to cytotoxic chemotherapy.
of 103 patients with amyloidosis without
Survival was significantly better for those patients with an LI
8 of the 13 had an LI > 0% (P = .015; χ² = 5.9) (Table 1).
Eleven (50%) of 22 patients with multiple myeloma and AL
responded to therapy (two of the 11 had an LI = 0%).
Thirteen (13%) of 103 patients with amyloidosis without
multiple myeloma responded to therapy (P < .001; χ² = 16.3).
Eight of the 13 had an LI = 0%. Currently, 82 of the
125 patients have died (36 of the 46 high LI patients and
46 of 79 LI = 0% patients). Survival was measured from the
time of biopsy-proven diagnosis of AL until death (Fig 3).
Survival was significantly better for those patients with an LI
of 0% (P = .02, log rank, and 0.03 Gehan-Wilcoxon). Because
the curves for LI ≥ 1 and 0 < LI < 1 are virtually
superimposable, the groups were combined into a group of 46
patients with LI > 0 for analysis. The median survival of all
patients with LI > 0 was 14.6 months, as compared with 29.8
months for those with LI = 0 (Table 1). In the high LI group,
the survival at 60 months is projected to be 20%; in the LI = 0
group, a survival of 29% at 60 months is projected. Among
responders to treatment, the median survival was 41.6
months, indicating a response to treatment is clinically
meaningful. Alternatively, response to treatment may reflect
an inherently good prognosis subset.
When the data from patients with or without myeloma are
compared rather than data from all patients with low or high
LI, the response distinctions blur. A disproportionate number
of the patients with increased LI had myeloma (Fig 2).
Nineteen myeloma-associated amyloid patients had LI > 0;
nine (47%) of these 19 patients responded. This response rate
is significantly better than the response of patients with AL
only, whether the LI was 0 or >0 (P < .05). When myeloma
patients are excluded from the response data, statistically
significant differences are lost. Of 27 AL-only patients with
LI > 0, five (19%) responded. There were eight responses
among the 76 amyloidosis patients with LI = 0 (11%;
P = .28). Survival analysis excluding those patients with
myeloma continued to show significant differences in sur-

dival between those with LI = 0 (30.9 months) and those
with LI > 0 (14.1 months; P < .05, Gehan-Wilcoxon) (Fig
4). The presence of myeloma does not appear to be a
 prognostic factor for survival. Survival of myeloma patients
was not inferior to that of patients who had only AL (17.6 v
25.5 months; P > .2). The adverse effect of LI on survival is
independent of the presence of myeloma, and the presence
of myeloma is not the most important prognostic factor; rather,
increased LI directly predicts survival. There were no clinical
differences between those patients without myeloma whose
LI was 0 and those whose LI exceeded 0. Specifically, no
differences in plasma cell percentages or morphology were
present.

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**Table 1. Response and Survival in Primary Systemic Amyloidosis**

<table>
<thead>
<tr>
<th>LI</th>
<th>Patients (n)</th>
<th>Responses</th>
<th>Response Rate (%)</th>
<th>Median Survival (Mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>79</td>
<td>10</td>
<td>13</td>
<td>29.8</td>
</tr>
<tr>
<td>0.2-0.8</td>
<td>32</td>
<td>8</td>
<td>25</td>
<td>14.6</td>
</tr>
<tr>
<td>≥ 1.0</td>
<td>14</td>
<td>6</td>
<td>43</td>
<td>17.6</td>
</tr>
</tbody>
</table>

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**Fig. 1. Distribution of LI values in 125 patients with AL.**

8 respondents, 1 responder.

**Fig. 2. Response rates of AL patients, with and without myeloma and by LI value.**

**Fig. 3. Survival of 125 patients with primary systemic AL.**

Thin solid line, survival among 79 patients with LI = 0; thick solid line, survival among 32 patients with 0 < LI < 1; broken line, survival among 14 patients with LI ≥ 1.
The LI is a useful technique for identifying cells in the DNA synthetic phase (S phase) of the cell cycle. A small fraction of most cell populations, normal and abnormal, is undergoing DNA synthesis at any time. In multiple myeloma, determining the fraction of those cells in S phase (ie, LI) is useful in distinguishing active myeloma from monoclonal gammapathy of undetermined significance and smoldering multiple myeloma. The LI also helps predict prognosis and survival. Recognition of plasma cells in the marrow can be difficult; confusion with erythroblasts and large lymphocytes may occur. To overcome the difficulty of morphologic identification of plasma cells, a simultaneous stain for cytoplasmic immunoglobulin is used. This permits recognition of all normal plasma cells, and with the use of fluorescence microscopy, the percentage of labeled cells can be easily determined.

AL is a plasma cell dyscrasia. More than 80% of AL patients have a detectable monoclonal protein in the serum or urine. The median number of marrow plasma cells seen in 198 patients with AL was 8%. The association of AL with multiple myeloma has been recognized for decades. When first diagnosed as having AL, 20% of patients have coexistent multiple myeloma. Recognizing multiple myeloma in the presence of AL is often difficult. A significant overlap exists between those patients with the malignancy and those with nonmalignant plasma cell dyscrasia. Clinical judgment often enters into the differentiation of the two syndromes. Simple inspection of the bone marrow is inadequate to determine which patients have myeloma. In a Mayo Clinic series, 20% of patients with multiple myeloma associated with AL had less than 10% plasma cells in their bone marrow. Conversely, 11% of AL patients without myeloma had an excess of 20% plasma cells in the marrow.

The presence of a serum M spike was not helpful in the differentiation. Of 171 AL patients, 41% had a recognizable band on serum protein electrophoresis, as compared with 36% who had myeloma and AL. Recognition of myeloma coexisting with AL is clinically important. Because clinicians are more likely to treat myeloma-associated AL with chemotherapy, the diagnosis of myeloma has significant treatment implications. In addition, there are important prognostic differences between the two groups. In a multivariate analysis, the presence of myeloma was recognized as having an independent and statistically significant adverse effect on survival after the first year. In multiple myeloma, the LI has significant predictive value. Myeloma patients with an increased LI tend to have a swift response to chemotherapy followed by quick relapse as the rapidly dividing cells quickly acquire resistance to chemotherapy. The higher the number of mitotic figures, the higher the probability that a chemotherapy-resistant cell will appear.

The current study was undertaken to address three questions. First, does the LI help distinguish those AL patients with myeloma from those who have only AL? Second, does the LI help predict response in patients treated with alkylating agents? Third, does the LI indicate overall survival in AL patients?

Of the AL patients, 18% had overt multiple myeloma. Nineteen myeloma plus AL patients (86% of myeloma patients) had LI > 0. Of patients with AL alone, 26% had a LI > 0. The LI is very useful in separating the subset of patients with myeloma. When response was assessed, 30% of patients with LI > 0 responded. When the LI was 0, 13% responded. There is a statistically significant survival advantage for the LI = 0 group. When myeloma patients are excluded from the analysis, the survival advantage of LI = 0 persists, indicating that the predictive value of the LI is independent of diagnosis. The presence of myeloma is not a more important prognostic factor than the LI in patients with AL (myeloma v AL alone; P > .2).

Bone marrow plasma cells may be proliferative or nonproliferative. Patients with monoclonal gammapathy of undetermined significance have low plasma cell proliferative activity, and the LI generally is 0. In multivariate analysis, the LI is an independent predicator of survival in multiple myeloma. In addition, a new staging system has been proposed for multiple myeloma that uses only the LI and the serum $\beta_2$-microglobulin level.

We believe that the survival disadvantage of a high LI in AL can be explained in two ways. First, there is a preponderance of myeloma-associated AL in this group. A previous report indicated that the median survival of AL patients exceeds that of myeloma and AL patients by 10.7 months. In our study, the presence of myeloma shortened median survival by 8 months, but this did not achieve statistical significance. A subsequent multivariate analysis revealed that coexistent myeloma adversely affected survival after the first year. Figures 3 and 4 reveal similar survival curves for the first year. After the first year, the curves diverge. Approximately 40% of AL patients die in the first year after diagnosis. After the first year, however, those who have coexistent myeloma begin to die of their malignancy. Myeloma patients with an increased LI tend to have rapid response but rapid relapse and early death. Median survival of one group of myeloma patients with an increased LI was 18 months. We believe that our data for AL patients are comparable and account for the higher response rate but shorter survival.
When the plasma cell LI is applied to primary AL, its usefulness is clear. First, an increased LI indicates a high likelihood of associated multiple myeloma. Second, an increased LI predicts a better response to alkylating agents, primarily because this group contains a disproportionate number with myeloma who respond more often but have a shorter survival. Because responders fare well (median survival, 41.6 months) and show clinically meaningful prolongation of survival, this is important in terms of treatment selection. Because patients with AL have previously been treated with noncytotoxic agents such as dimethyl sulfoxide (DMSO) and colchicine, there are important therapeutic implications. We infer that a high LI reflects a proliferative plasma cell population. In these instances, one must strongly consider the use of cytotoxic agents in the treatment of this subset of patients with AL (41% of whom will have overt coexistent myeloma). It appears that in this group, DMSO, colchicine, or other noncytotoxic agents are contraindicated. Finally, an increased LI reflects poor prognosis (median survival, 14.1 months and 30.9 months for those with LI > 0 and LI = 0, respectively). The significance of this persists even when patients with myeloma (median survival, 17.6 months) are excluded from survival analysis.

REFERENCES

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