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 (BrdU) to label plasma cells in patients with AL and myeloma. 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.4 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.15 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 \( \chi^2 \) 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 10\(^6\) cells were incubated with 10 \( \mu \)mol/L bromodeoxyuridine (BrdU) and 1.0 \( \mu \)mol/L fluorodeoxyuridine. Cytocentrifuge slides were prepared, air dried, and fixed in 95% ethyl alcohol. Mouse antibrumodeoxyuridine (BU-1) (20 \( \mu \)g) 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 antimouse IgG (8 \( \mu \)g). Plasma cells were also identified by immunofluorescence microscopy. Fluorescein isothiocyanate (FITC)-labeled antihuman \( \kappa \) or antihuman \( \lambda \) 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 \( v \) three (4%) of 79 patients with LI = 0% (\( P < .0001; \chi^2 = 28.2 \)). 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.
Thirteen (13%) of 103 patients with amyloidosis without myeloma 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 = .015; \( \chi^2 = 5.9 \)) (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; \( \chi^2 = 16.3 \)). 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 survival 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.
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 gammopathy 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 gammopathy of undetermined significance have low plasma cell proliferative activity, and the LI generally is 0. In multivariate analysis, the LI is an independent predictor 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 β2-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 nontoxic 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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