Value of $\beta_2$-Microglobulin Level and Plasma Cell Labeling Indices as Prognostic Factors in Patients With Newly Diagnosed Myeloma


Beta2-microglobulin ($\beta_2$M), a low-molecular weight protein found on the surface of all nucleated cells, is the light chain of the HLA-A, -B, and -C histocompatibility antigen complex. $\beta_2$M can be recovered from lymphocyte cultures that have been stimulated with mitogens and from myeloma cells in culture. Serum $\beta_2$M levels are often increased in patients with multiple myeloma (MM) and also in renal insufficiency. Renal insufficiency may contribute to the increase in $\beta_2$M in some myeloma patients. It is not entirely clear whether nonrenal-related increases are due to myeloma cells or to normal cells. Studies correlating $\beta_2$M levels with myeloma stage and with disease status and survival suggest that $\beta_2$M may be a product of myeloma cells and can be used as a tumor marker to predict the course of the disease.

The plasma cell labeling index (LI) is the percentage of plasma cells that synthesize DNA; LI serves as a measure of myeloma cell proliferation. The LI has been used to distinguish overt progressive myeloma from stable monoclonal gammapathies that do not require treatment, such as monoclonal gammopathy of undetermined significance and smoldering multiple myeloma. Because the LI identifies patients with more aggressive myeloma, it is also useful as a prognostic indicator.

The investigators initiated this study to determine the value of $\beta_2$M level, LI, and other variables as predictors of outcome in patients with MM.

**MATERIALS AND METHODS**

**Patient Selection**

The patients examined in this study were the same cohort as in an earlier study of morphologic features but with additional follow-up. Between March 1978 and February 1982, 100 patients were studied just prior to initial chemotherapy for MM; all were followed for at least 3.5 years. Sufficient information was available on all patients to allow clinical staging, and bone marrow plasma cell LI were determined in all. The available laboratory data in all cases included results of bone marrow examination, metastatic bone survey, serum protein electrophoresis and immunoelectrophoresis or immunofixation, and determination of serum calcium, creatinine, and hemoglobin levels. Urine protein electrophoresis and immunoelectrophoresis or immunofixation were performed in 96 patients. Patients with monoclonal gammapathy of undetermined significance or smoldering multiple myeloma were excluded from this study.

**METHODS**

The following variables were examined: age, sex, monoclonal immunoglobulin type, myeloma clinical stage, type of treatment and response to treatment, serum creatinine and calcium levels, and plasmablastic subtype. The median age at diagnosis of the 100 patients (male-to-female ratio, 1.5:1.0) in this series was 63 years. Clinical stage by the Durie-Salmon system was distributed as follows: IA, 7%; II, 58%; III, 12%; IIIB, 11%; IIIA, 11%; IIIB, 12%. Renal insufficiency (serum creatinine > 2 mg/dL) was present in 24% of the patients, and hypercalcemia (serum calcium > 11 mg/dL) was present in 15%.

Patients were classified as stage III if they had extensive lytic bone lesions. The presence of four or more bone lesions, widely used by most groups as a criterion for stage III disease, was not useful in our hands: with this criterion more than 50% of MM patients were stage III, but there was no difference in survival between stage III patients and stage I and II patients. However, there was a significant difference with the criterion of extensive lytic bone lesions (unpublished data).

Serum electrophoresis and immunoelectrophoresis, performed in all patients, identified the following monoclonal proteins: IgG, 49%; IgA, 31%; IgD, 3%; and light chain only, 6%. Of the serum monoclonal proteins, 58% were $\kappa$ and 42% were $\lambda$. Ten percent of the patients had no monoclonal protein identifiable in the serum.

Urine protein electrophoresis and immunoelectrophoresis, performed in 96 patients, identified free urine monoclonal light chains...
in 76 patients: 57% and 43% λ. Twenty patients had no monoclonal free light chain in the urine. All patients had a monoclonal protein, either in the serum or in the urine.

Plasma cell LI were measured in all 100 patients and ranged from 0.0% to 7.0% (median, 0.6%). The LI was <0.2% in 46% of patients and ≥0.4% in 54% of patients. The distribution of morphologic subtypes was mature, 28%; intermediate, 38%; immature, 19%; and plasmablastic, 15%.

Initial therapy consisted of melphalan and prednisone in 81% of patients. Nineteen percent received other chemotherapy such as melphalan intravenously (IV) with prednisone orally or in combination with cyclophosphamide, N,N-bis(2-chloroethyl)-N-nitrosourea (BCNU), and prednisone, with or without doxorubicin (Adriamycin).

**β2M Levels**

Serum samples were retrieved retrospectively from a serum bank. β2M levels were measured by an enzyme-linked immunosorbent assay using the Phadezym β2-micro test kit (Pharmacia Diagnostics, Uppsala, Sweden). The mean value in 20 normals increased slightly with age (20 to 60 years), but the upper limit of normal was 2.7 μg/mL (mean ± 2 SD), regardless of age.

The β2M values (μg/mL) for patients with increased serum creatinine (≥1.5 mg/dL) were “corrected” for serum creatinine level by the method of Garewal et al.22

**Plasma Cell LI**

These were measured by using a high-speed autoradiography method23 with modifications.24 After bone marrow cells were incubated with high-specific-activity [3H]thymidine, slides were prepared and subjected to autoradiography and Wright’s staining. The LI was determined by counting 500 plasma cells and was reported as the percentage of cells noted autoradiographically to be labeled.

**RESULTS**

**β2M Levels**

Relationship to serum creatinine. The mean serum β2M level in 84 normal volunteers was 1.6 μg/mL (2 SD range, 0.7 to 2.7 μg/mL). Patients’ β2M levels ranged from 1.4 to 34.7 μg/mL (mean, 5.7 μg/mL); 45 had β2M ≥ 4 μg/mL and 26 had β2M > 6 μg/mL. Values for β2M corrected for serum creatinine ranged from 0.8 to 17.9 μg/mL (mean ± SD, 3.5 ± 2.1). The uncorrected data were used for the subsequent analyses. With a discriminant of 6 μg/mL, only 7% of patients with creatinine <2 mg/dL (five patients) had a high β2M. With a discriminant of 4 μg/mL, 29% with creatinine <2 mg/dL had a high β2M. The investigators used both the continuous variable and the discriminant of 4 μg/mL for the proportional hazards model analysis in this study. The distribution of β2M levels relative to serum creatinine levels is shown in Table 1.

Relationship to other prognostic factors. The investigators found a positive correlation between the uncorrected serum β2M value and several other prognostic factors (Table 1). In particular there was a greater frequency of increased β2M levels among patients with increased creatinine, advanced stage, plasmablastic morphology, or age >63 years. There was very little difference in β2M level among patients with low or high LI.

**Univariate Survival Analysis**

The median survival of the 100 patients measured from the beginning of initial chemotherapy was 26 months. The univariate proportional hazards analysis ranked the variables in the following order of significance in predicting survival: age, β2M level, stage, LI, creatinine, and plasmablastic morphology (Table 2). The corrected β2M value was less useful than the uncorrected value as a discriminant of poor survival because the high value for β2M encountered in patients with high creatinine (≥2 mg/dL) became normal (<4 μg/dL) in all instances (data not shown).

Serum albumin level (divided at the median, 3.7 g/dL) was not related to survival differences. The group of patients with very low albumin values was very small and was not examined. These results confirm those of Kyle and Elveback.25

Patients with β2M ≥ 4 μg/mL had a poorer survival than patients with low β2M (Fig 1). With a discriminant of 6 μg/mL, the median survivals of patients with high and low β2M were slightly less well separated, 12 and 37 months. Patients with stage IIB or III MM had a poorer survival than patients with stage I or IIA (Table 2). Patients with LI ≥ 0.4% had a poorer survival than patients with low LI (Fig 2). Patients with creatinine ≥2 mg/dL had a poorer survival.

**Table 1. Relationship of Increased Uncorrected β2M Values to Other Prognostic Factors**

<table>
<thead>
<tr>
<th>Factor</th>
<th>n Values</th>
<th>% with β2M ≥ 4 μg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine</td>
<td>76 &lt;2 mg/dL</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>24 ≥2 mg/dL</td>
<td>96</td>
</tr>
<tr>
<td>Stage</td>
<td>85 I and II A</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>35 III and III</td>
<td>80</td>
</tr>
<tr>
<td>Morphology</td>
<td>85 Nonplasmablastic</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>15 Plasmablastic</td>
<td>87</td>
</tr>
<tr>
<td>Age</td>
<td>50 &lt;63 yr</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>50 &gt;63 yr</td>
<td>62</td>
</tr>
<tr>
<td>LI</td>
<td>46 Low</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>54 High</td>
<td>46</td>
</tr>
</tbody>
</table>

Univariate Proportional Hazards Analysis of Prognostic Factors

<table>
<thead>
<tr>
<th>Factor</th>
<th>P</th>
<th>Median Survival (mo)</th>
<th>Discirninant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>&lt;.0001*</td>
<td>42 &lt;63 yr</td>
<td>&lt;4 μg/mL</td>
</tr>
<tr>
<td>β2M (uncorrected)</td>
<td>&lt;.0001*</td>
<td>43 &lt;4 μg/mL</td>
<td>≥4 μg/mL</td>
</tr>
<tr>
<td>Stage</td>
<td>&lt;.001†</td>
<td>32 I and II A</td>
<td>16 III and III</td>
</tr>
<tr>
<td>LI</td>
<td>&lt;.01†</td>
<td>38 &lt;0.4%</td>
<td>18 ≥0.4%</td>
</tr>
<tr>
<td>Creatinine</td>
<td>&lt;.03†</td>
<td>34 &lt;2 mg/dL</td>
<td>14 ≥2 mg/dL</td>
</tr>
<tr>
<td>Morphology</td>
<td>&lt;.05†</td>
<td>29 Nonplasmablastic</td>
<td>10 Plasmablastic</td>
</tr>
</tbody>
</table>

*Probability of these groups being different by log-rank analysis of continuous variable.
†Probability based on log-rank analysis of discriminant.
than those with creatinine <2 mg/dL (Table 2). Finally, patients with plasmablastic myeloma had a poorer survival than those who had nonplasmablastic myeloma (Table 2).

**Multivariate Survival Analysis**

In the multivariate proportional hazards model, the ranking of variables predicting survival was as follows: age, uncorrected β₂M (discriminant, 4 μg/mL), and plasma cell LI (discriminant, 0.4%; Table 3). Stage, creatinine, and plasmablastic subtype no longer predicted outcome; only LI functioned as a significant prognostic factor after age and serum β₂M were considered.

Among patients over age 63, those with β₂M levels ≥4 μg/mL had a median survival of only 10 months compared with 34 months for patients with low β₂M values; among patients under age 63, those with β₂M ≥4 μg/mL had a median survival of 18 months compared with 53 months for those with β₂M < 4 μg/mL. Among patients with high β₂M values, those with high LI (≥0.4%) had a median survival of 11 months compared with 17 months for those with low LI; among those with low β₂M values, those with high LI had a median survival of 29 months compared with 48 months for those with low LI (the longest survival in any combination of variables found in this study, except for those with age <63 years and low β₂M).

**Effects of β₂M After Consideration of Creatinine**

Twenty-three of the 24 patients with increased serum creatinine (>2 mg/dL) also had serum β₂M ≥4 μg/mL; these 24 patients had a median survival of 14 months. With the proportional hazards model, even after consideration of the serum creatinine, β₂M level retained its prognostic value. In patients with creatinine <2 mg/dL, those with β₂M ≥4 μg/mL had a median survival of only 11 months compared with 46 months for those with β₂M < 4 μg/mL (Fig 3).

### Prognostic Groups by Using β₂M and LI

By using the principal prognostic factors identified in this study it was possible to divide MM patients into three risk groups: (1) low risk (low β₂M [<4 μg/mL] and low LI [<0.4%; n = 30], median survival 48 months); (2) intermediate risk (low β₂M and high LI [≥0.4%; n = 25], median survival 29 months); and (3) high risk (high β₂M [n = 45], median survival 12 months; Fig 4).

### DISCUSSION

The importance of β₂M and LI in predicting survival in MM patients is relevant to clinical practice and clinical trials. Increased use of intensive chemotherapy regimens and autologous and allogeneic bone marrow transplantation makes it increasingly important to identify different prognostic groups.

In patients with MM, β₂M has been confirmed as a highly significant prognostic factor in each study in which it has been examined. Several investigators have recently shown the independent prognostic superiority of β₂M levels. This is an important issue because of the ready availability of other indicators such as stage, creatinine level, morphologic features, and proliferative status of the bone marrow myeloma cells. Despite these clear

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Table 3. Multivariate Proportional Hazards Analysis of Prognostic Factors

<table>
<thead>
<tr>
<th>Factor</th>
<th>P for Survival Differences</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>After Adjustment</td>
</tr>
<tr>
<td></td>
<td>for Age</td>
</tr>
<tr>
<td>β₂M</td>
<td>.001</td>
</tr>
<tr>
<td>LI (discriminant, 0.4%)</td>
<td>.01</td>
</tr>
<tr>
<td>Stage (I and IIA vs III)</td>
<td>.03</td>
</tr>
<tr>
<td>Creatinine (discriminant, 2 mg/dL)</td>
<td>.03</td>
</tr>
</tbody>
</table>

*P = .08 with the continuous variable.
indicators of the prognostic value of serum $\beta_2$M levels in MM, controversy has arisen because of the suggestion that $\beta_2$M level does not improve on the use of stage and creatinine level.\textsuperscript{28,30} In the investigators’ study, an uncorrected $\beta_2$M level >4 $\mu$g/mL identified patients with poorer survival than creatinine level, stage, and the presence of plasmablastic morphology. There was little relationship between $\beta_2$M level and LI. Of all the variables considered, after age and $\beta_2$M, only LI had a significant relationship with survival.

Similar results showing the prognostic superiority of serum $\beta_2$M levels and independence from creatinine level were found in several other studies. Battaile et al.\textsuperscript{12} using univariate and multivariate analysis methods, demonstrated that the relationship of $\beta_2$M to survival was better than that of creatinine. Most other studies have also shown that serum $\beta_2$M is superior to serum creatinine as a prognostic variable.\textsuperscript{8,9,15,27}

Only two studies have suggested that the relationship of $\beta_2$M to survival is limited to its measurement of renal function.\textsuperscript{16,28,30} One of these studies\textsuperscript{30} was of a group of patients with a median survival much longer than expected for patients with MM: 65 months for patients with low serum $\beta_2$M and 25 months for those with high $\beta_2$M levels. There was probably a disproportionate number of good-risk patients in this study. In addition, a very low discriminant value for $\beta_2$M level was used, 2.9 $\mu$g/mL. All other studies have shown the difference between good-risk and poor-risk patients with higher discriminant levels of $\beta_2$M. Another study\textsuperscript{28} alleged that $\beta_2$M was of no prognostic value in patients with normal renal function. However, the discriminant for $\beta_2$M was set high, 7.6 $\mu$g/mL, and only six of 73 patients with normal renal function had high $\beta_2$M in this study. Setting the discriminant for serum $\beta_2$M too high or too low alters the impact of serum $\beta_2$M level on survival.

The investigators’ results are in agreement with those of most other studies that have shown a good correlation of $\beta_2$M level with Durie-Salmon myeloma stage (Table 1). In the investigators’ study the $\beta_2$M level was a better predictor of survival than stage when the proportional hazards model was used.

The plasma cell LI is a highly significant prognostic factor in MM.\textsuperscript{19,20} To date no published studies have addressed the prognostic value of the plasma cell LI in relationship to $\beta_2$M level. In the investigators’ study, plasma cell LI retained its ability to predict survival even after consideration of age and $\beta_2$M level. Of the combinations of two variables examined in this study, low LI and low $\beta_2$M were associated with the longest survival (median, 48 months), except for the combination of low $\beta_2$M and age >63 years (median survival, 53 months).

In this study of 100 patients with MM, age, $\beta_1$M level, and LI were the three most important factors predicting survival. Age is important to consider before treatment is begun, both in clinical practice and in group trials. Although patients over the age of 63 years have poorer survival, they are least able to sustain more intensive therapy. Thus, identification of a subset at higher risk among older patients may not lead to a change in treatment.

In younger patients increased $\beta_2$M level and LI are the most important risk factors. The relatively longer median survival, >4 years, in young patients with low $\beta_2$M may be considered before an intensive therapy program is implemented. Younger patients who, in addition, have low LI have a remarkably long survival, median >5 years. The risk–benefit ratio for very intensive therapy is unacceptably high for the younger low-risk patients; predictably safe and more effective treatments are needed. Conversely, high-risk young patients may be considered for a more aggressive treatment approach.

Although other features such as stage, creatinine value, and plasmablastic morphology are confirmed as having prognostic value, they are dependent variables compared with $\beta_2$M and LI. $\beta_2$M currently is in widespread use, and LI is clinically available.\textsuperscript{11} $\beta_2$M and LI should be measured as part of pretreatment evaluation for prognostic assessment and for MM treatment trials.

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