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

By Philip R. Greipp, Jerry A. Katzmann, W. Michael O'Fallon, and Robert A. Kyle

$\beta_2$-microglobulin ($\beta_2$M) has been proposed as a prognostic factor in multiple myeloma (MM), but $\beta_2$M levels are reported to correlate with other prognostic indicators such as stage and creatinine level. This study addressed the independent prognostic values of these and other variables, including plasma cell labeling indices (LI), in patients with newly diagnosed MM. $\beta_2$M levels were measured with an enzyme-linked immunosorbent assay. LI were determined with a $[3H]$thymidine autoradiography method. By multivariate analysis and Kaplan-Meier survival analysis, the uncorrected $\beta_2$M level remained the most significant prognostic factor after adjustment for age. Stage and creatinine level were closely related to $\beta_2$M level and were no longer predictive of outcome after adjustment for age and $\beta_2$M. Plasma cell LI varied independently of $\beta_2$M level and remained predictive. A subset of patients with plasmablastic myeloma had poor survival since $\beta_2$M level and plasma cell LI were high. By using $\beta_2$M level and LI, three risk groups were defined: low ($\beta_2$M < 4 $\mu$g/mL and LI < 0.4%, median survival 48 months); intermediate ($\beta_2$M < 4 $\mu$g/mL and LI ≥ 0.4%, median survival 29 months); and high ($\beta_2$M ≥ 4 $\mu$g/mL, median survival 12 months). Such grouping may better identify MM patients who might benefit from new treatment regimens.

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in 76 patients: 57% κ 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.¹⁰

**Plasma Cell LI**

These were measured by using a high-speed autoradiography method¹¹ with modifications.¹² After bone marrow cells were incubated with high–specific-activity [³H]thyminde, 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.

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

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

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.²⁶

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 2. Univariate Proportional Hazards Analysis of Prognostic Factors

<table>
<thead>
<tr>
<th>Factor</th>
<th>P</th>
<th>Median Survival (mo)</th>
<th>Discriminant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>&lt;.0001*</td>
<td>42</td>
<td>&lt;63 yr</td>
</tr>
<tr>
<td>β₂M (uncorrected)</td>
<td>&lt;.0001*</td>
<td>43</td>
<td>&lt;4 μg/mL</td>
</tr>
<tr>
<td>Stage</td>
<td>.007†</td>
<td>32</td>
<td>I and II</td>
</tr>
<tr>
<td>LI</td>
<td>.02†</td>
<td>38</td>
<td>&lt;0.4%</td>
</tr>
<tr>
<td>Creatinine</td>
<td>.03†</td>
<td>34</td>
<td>&lt;2 mg/dL</td>
</tr>
<tr>
<td>Morphology</td>
<td>.05†</td>
<td>29</td>
<td>Nonplasmablastic</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 $\beta_2$M (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 $\beta_2$M were considered.

Among patients over age 63, those with $\beta_2$M levels ≥4 µg/mL had a median survival of only 10 months compared with 34 months for patients with low $\beta_2$M values; among patients under age 63, those with $\beta_2$M ≥4 µg/mL had a median survival of 18 months compared with 53 months for those with $\beta_2$M < 4 µg/mL. Among patients with high $\beta_2$M 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 $\beta_2$M 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 $\beta_2$M).

**Effects of $\beta_2$M After Consideration of Creatinine**

Twenty-three of the 24 patients with increased serum creatinine (≥2 mg/dL) also had serum $\beta_2$M ≥ 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, $\beta_2$M level retained its prognostic value. In patients with creatinine <2 mg/dL, those with $\beta_2$M ≥ 4 µg/mL had a median survival of only 11 months compared with 46 months for those with $\beta_2$M < 4 µg/mL (Fig 3).

**Prognostic Groups by Using $\beta_2$M 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 $\beta_2$M [≤4 µg/mL] and low LI [≤0.4%; n = 30], median survival 48 months); (2) intermediate risk (low $\beta_2$M and high LI [≥0.4%; n = 25], median survival 29 months); and (3) high risk (high $\beta_2$M [n = 45], median survival 12 months; Fig 4).

**DISCUSSION**

The importance of $\beta_2$M 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, $\beta_2$M 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 $\beta_2$M 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
indications 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. In the investigators' study, an uncorrected $\beta_2$-M level $\geq 4 \mu g/mL$ 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. Bataille et al. using univariate and multivariate analysis methods, demonstrated that the relationship of $\beta_2$-M to survival was closer than that of creatinine. Most other studies have also shown that serum $\beta_2$-M is superior to serum creatinine as a prognostic variable. Only two studies have suggested that the relationship of $\beta_2$-M to survival is limited to its measurement of renal function. One of these studies 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 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. 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, $\beta_2$-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 should 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 factors 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. $\beta_2$-M and LI should be measured as part of pretreatment evaluation for prognostic assessment and for MM treatment trials.

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Value of beta 2-microglobulin level and plasma cell labeling indices as prognostic factors in patients with newly diagnosed myeloma

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