Multiple Myeloma: Significance of Plasmablastic Subtype in Morphological Classification

By Philip R. Greipp, Nancy M. Raymond, Robert A. Kyle, and W. Michael O’Fallon

We classified 100 cases of myeloma before chemotherapy as mature (28), intermediate (38), immature (19), or plasmablastic (15). The plasmablastic group had an estimated median survival (Kaplan-Meier method) of ten months, compared to 35 months for the other types (P < .05). Decreased survival in the plasmablastic group was due to more frequent deaths in the first six months. There were no significant differences in survival among the mature, intermediate, and immature groups or among patients with different morphological grade or asynchrony scores. The plasmablastic myeloma group had more frequent renal insufficiency and higher plasma cell labeling indices, which may have contributed to the shorter survival.

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Although morphological classification schemes are important as a guide to outcome in other malignancies, an acceptable system for myeloma has not yet been developed. This article reports the application of a morphological classification in 100 cases of multiple myeloma. We report survival analysis and relationship to other variables affecting outcome in myeloma, including serum creatinine and plasma cell labeling index.

Materials and methods

Patient Selection

One hundred patients were studied between March 1978 and February 1982 before chemotherapy for multiple myeloma. This allowed at least one year of follow-up for all patients. Only patients for whom sufficient information was available to allow clinical staging and cell kinetic studies were entered. The laboratory procedures included bone marrow examination, metastatic bone survey, serum and urine protein electrophoresis and immunoelectrophoresis, and determination of serum calcium, creatinine, and hemoglobin levels and bone marrow plasma cell labeling index. Urine electrophoresis and immunoelectrophoresis were performed on 96 patients. Patients with monoclonal gammopathy of undetermined significance (MGUS) or smoldering multiple myeloma (SMM) were excluded from this study.

Myeloma Classification System

We classified plasma cells as mature, intermediate, immature, or plasmablastic according to defined criteria (Table 1 and Fig 1). After a 200-cell differential count by one of us (N.M.R., done without knowledge of the patient’s course), we determined the myeloma subtype based on the proportion of the various types of plasma cells (Table 2 and Fig 2). We assessed reproducibility of the subtyping system by using 30 coded slides containing examples of all subtypes. We determined plasma cell grade and asynchrony scores by methods previously described.

Other Studies

We selected the following variables at the beginning chemotherapy for further analysis: age, monoclonal immunoglobulin type, myeloma clinical stage, type of treatment, response to treatment, renal insufficiency (creatinine >2 mg/dL), hypercalcemia (calcium >11 mg/dL), Bence Jones protein excretion, and plasma cell labeling indices.

Plasma cell labeling indices. The plasma cell labeling index was determined by a high-speed autoradiography method with modifications. After bone marrow cells were incubated with high-specific activity [\(^3\)H]thymidine, slides were prepared and subjected to autoradiography and Wright’s staining. We determined the labeling index (percentage of plasma cells labeled) by counting 500 plasma cells.

Response to Treatment

We defined objective responses as greater than 50% decrease in serum or urine monoclonal protein (if urine monoclonal protein was greater than 1 g/24 h). We required a decrease in the urine monoclonal protein to less than 300 mg/24 h if the value was less than 1 g/24 h initially. A decrease in hemoglobin, an increase in creatinine or calcium, or an increase in bone marrow plasma cells or in size and number of bone lesions disqualified as an objective response.

Statistical Analysis

Survival was measured from the date of the beginning of chemotherapy. Survival curves were constructed by using the Kaplan-Meier method and were compared by using the Gehan-Wilcoxon test, the log-rank test, and the likelihood ratio test. In addition, curves were compared after adjustment for covariates by applying the proportional hazards model.

Results

The clinical, morphological, and cell kinetic data from these 100 patients with untreated symptomatic multiple myeloma were obtained no more than two months before chemotherapy was begun. Median age was 63 years. Male-female ratio was 3:2. Estimated median survival of the whole group was 28 months (Kaplan-Meier method).

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Clinical Data

Clinical stages according to the Durie-Salmon clinical staging system were as follows: IA, 7%; IIA, 58%; IIB, 12%; IIIA, 11%; and IIIB, 12%. Before treatment, renal insufficiency (creatinine >2 mg/dL) was present in 24% of patients, and hypercalcemia (calcium >11 mg/dL) was present in 15%.

Serum and Urine Electrophoresis and Immunoelectrophoresis

Serum electrophoresis and immunoelectrophoresis were performed in all patients and identified the following monoclonal protein isotypes: IgG, 49%; IgA, 31%; IgD, 3%; biclonal, 1%; and light chain only, 16%. Light chain typing of the whole monoclonal immunoglobulin fraction revealed 58% as λ and 42% as κ.

We performed urine protein electrophoresis and
immunoelectrophoresis in 96 patients. Free urine monoclonal light chain was identified in 76 patients: 57% κ and 43% λ. In 20 patients, there was no free light chain in the urine.

**Plasma Cell Labeling Indices**

These ranged from 0.0% to 7.0% (median 0.6%). The labeling index was greater than 0.0% in 76% of patients and was greater than 1% in 27% of patients.

**Treatment and Response to Treatment**

Eighty-one percent of patients received melphalan and prednisone as initial therapy. Nineteen percent received other chemotherapy, such as intravenous melphalan with oral prednisone or in combination with cyclophosphamide, N,N-bis(2-chloroethyl)-N-nitrosourea (BCNU), and prednisone, with or without hydroxydaunorubicin. Infrequently, other combinations were used, including cyclophosphamide, vincristine, and prednisone and melphalan, vincristine, and prednisone. Among 85 patients evaluable, we documented an objective response to treatment (see Materials and Methods) in 54%.

**Morphological Assessment**

Using the morphological classification scheme in Fig 2 and Table 2, we identified the frequency of myeloma types as follows: mature, 28%; intermediate, 38%; immature, 19%; and plasmablastic, 15%. The proportion of plasmablasts in the plasmablastic type ranged from 2% to 9% (median, 3%). Proportions of plasma cell types in each of the classifications are listed in Table 3. N.M.R. and P.R.G. independently reviewed 30 coded slides containing examples of all subtypes. Of the cases originally classified as plasmablastic, a high proportion were identified as plasmablastic on the basis of the coded slides: 83% for N.M.R. and 100% for P.R.G. Overall concordance between P.R.G. and N.M.R. was 77%. Differences were not limited to specific types of myeloma. For the plasmablastic subtype, concordance was 79%.

**Survival by Morphological Subtype**

Estimated median survival (Kaplan-Meier method) for the 15 patients with plasmablastic myeloma was ten months (Fig 3). Only 2 of 15 (13%) were living at last follow-up. In contrast, estimated median survival for the other subtypes combined was 35 months. In this group, 41 of 85 (48%) were living at last follow-up. Survival differences between the plasmablastic and other types were significant by the Gehan-Wilcoxon test (P < .05). This test recognizes early differences in survival and reflects a high frequency of deaths in the first six months in the plasmablastic group (Table 4). When the log-rank and likelihood ratio tests were used to compare the same two survival curves, the differences were not significant (P = .10 and .11, respectively). There were no significant differences in survival among the mature, intermediate, and immature groups.

Careful visual inspection of the data and the application of the proportional hazards model led us to the conclusion that there were no significant differences in survival among patients with different values for other morphological parameters, such as nuclear and nucleolar sizes and grade and asynchrony scores.

**Relationship of Morphological Classification to Other Variables**

Age distribution, type of treatment, and objective response rate were similar for all morphological types. Distribution of clinical stage (Table 5) and of mono-

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**Table 3. Proportions (%) of Myeloma Cell Types**

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Plasmablastic Myeloma</th>
<th>Immature Myeloma</th>
<th>Intermediate Myeloma</th>
<th>Mature Myeloma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mature</td>
<td>2.7 ± 3.0 (0-9)</td>
<td>3.1 ± 3.7 (0-9)</td>
<td>4.1 ± 3.7 (0-9)</td>
<td>25.8 ± 15.9 (11-67)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>72.8 ± 11.3 (49-90)</td>
<td>72.2 ± 10.4 (37-85)</td>
<td>89.4 ± 5.6 (69-99)</td>
<td>70.7 ± 14.3 (33-87)</td>
</tr>
<tr>
<td>Immature</td>
<td>20.9 ± 11.2 (4-46)</td>
<td>25.3 ± 12.2 (13-63)</td>
<td>5.8 ± 3.5 (0-12)</td>
<td>3.5 ± 3.3 (0-12)</td>
</tr>
<tr>
<td>Plasmablastic</td>
<td>3.9 ± 2.3 (2-9)</td>
<td>0.2 ± 0.5 (0-1)</td>
<td>0.2 ± 0.4 (0-1)</td>
<td>0.1 ± 0.3 (0-1)</td>
</tr>
</tbody>
</table>

*Results are shown as mean ± SD, with range in parentheses.
clonal immunoglobulin heavy chain type were similar for all morphological groups. However, there was a difference in the \(\kappa/\lambda\) ratio among groups (Table 5). IgA monoclonal proteins were found in 7 of 15 patients with plasmablastic myeloma. Extramedullary extension of bone disease was found in five patients (four with IgA and one with \(\kappa\) Bence Jones protein only); these five had spinal involvement with cord compression (in two), sacral destruction with a soft tissue mass (in one), a maxillary mass (in one), and a right scapular soft tissue mass (in one).

Renal insufficiency and light chain excretion greater than 1 g/24 h were seen more frequently in the plasmablastic group. Frequency of hypercalcemia was also slightly more in the plasmablastic group: four of 15 (27%) compared to 11 of 85 (13%) for other types. Plasma cell labeling index greater than or equal to 1% was more frequent in the plasmablastic type: seven of 15 (47%) compared to 24% for other types (Table 5).

The proportional hazards model was used to compare survival in the plasmablastic group with the survivals in the other groups combined, with adjustment for increased serum creatinine value and plasma cell labeling index (an “adjusted” log-rank test). There was no significant difference in survival between the plasmablastic group and the other groups in this analysis (\(P = .21\)). Both increased serum creatinine concentration and plasma cell labeling index were significantly \(P < .05\) correlated with survival, whereas increased urinary light chain excretion was not and was not included in the model.

**DISCUSSION**

Demonstration of a morphological classification predicting survival in multiple myeloma would represent an important advance. Plasmablastic myeloma, as described in this study, has a median predicted survival of ten months from the beginning of chemotherapy compared to 35 months for the mature, intermediate, and immature types \((P < .05, \text{ Gehan-Wilcoxon test})\) (Fig 3). There were no significant differences in survival among the mature, intermediate, and immature types.

Although some attempts to identify a morphological subset of myeloma with a poorer prognosis have failed, several studies have shown results similar to ours (Table 6). Wutke et al\(^7\) reported results of a review of bone marrow aspirates and biopsies from 202 patients with multiple myeloma. They identified 32 patients (15%) as having plasmablastic morphology, by virtue of having more than 50% plasmablasts. The median survival of this group was 9.8 months, compared to 39.7 months for the plasmacytic type (<15% plasmablasts) and 16.1 months for the mixed subtype (20% to 50% plasmablasts). Tests of statistical significance and specific data about interobserver agreement were not reported. Interestingly, they noted a slightly increased frequency of extramedullary involvement in patients with plasmablastic myeloma. We found extramedullary extension of bone disease in five of 15 patients with plasmablastic myeloma.

In 1982, Bartl et al\(^8\) reported morphological observations (bone marrow biopsies) in 220 cases of myeloma. After independent review by three observers (without knowledge of patient identity or clinical status), they classified 71 cases (32%) as plasmablastic based on the presence of cells with “clear round nuclei, usually with single central nucleoli and variable amounts of basophilic cytoplasm and occasional perinuclear pale zone.” Plasmablastic patients survived a shorter time (19 months) than mature myeloma patients (41 months). Specific data about reproducibility and interobserver agreement were not reported. Patients with plasmablastic features more frequently had a high mitotic rate or total marrow replacement. As in the current study, they had renal insufficiency more frequently.

### Table 4. Estimated Death Rate in Plasmablastic Myeloma and Mature, Intermediate, and Immature Myeloma Combined

<table>
<thead>
<tr>
<th>Myeloma Type</th>
<th>Estimated Percent Dying</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>By 6 mo</td>
</tr>
<tr>
<td>Plasmablastic</td>
<td>40</td>
</tr>
<tr>
<td>Other types</td>
<td>9</td>
</tr>
</tbody>
</table>

### Table 5. Relationship of Myeloma Type to Other Prognostic Variables

<table>
<thead>
<tr>
<th>Myeloma Type</th>
<th>Stage</th>
<th>Serum Creatinine* &gt;2 mg/dL</th>
<th>Urine Light Chain &gt;1 g/24 h</th>
<th>Plasma Cell Labeling Index* &gt;1%</th>
<th>(k/\lambda)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mature</td>
<td>28</td>
<td>4</td>
<td>20</td>
<td>25</td>
<td>28</td>
</tr>
<tr>
<td>Intermediate</td>
<td>38</td>
<td>8</td>
<td>24</td>
<td>28</td>
<td>23†</td>
</tr>
<tr>
<td>Immature</td>
<td>19</td>
<td>11</td>
<td>16</td>
<td>26†</td>
<td>26</td>
</tr>
<tr>
<td>Plasmablastic</td>
<td>15</td>
<td>7</td>
<td>27</td>
<td>53</td>
<td>47</td>
</tr>
</tbody>
</table>

*Significant correlation with survival \((P < .05)\).
†Four patients did not have 24-hour urine protein electrophoresis prior to treatment.
Table 6. Summary of Morphological Subtyping in Myeloma

<table>
<thead>
<tr>
<th>Reference</th>
<th>No. of Cases</th>
<th>Bone Marrow</th>
<th>Types, No.</th>
<th>Criteria</th>
<th>Median Survival</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>41</td>
<td>Sternal aspirate</td>
<td>Grade 1, 7</td>
<td>Cells virtually all mature</td>
<td>&gt;2 yr</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Grade 2, 24</td>
<td>Intermediate maturity</td>
<td>Variable</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Grade 3, 10</td>
<td>Very immature cells present</td>
<td>&lt;1 yr</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>202</td>
<td>Bone marrow aspirate and/or biopsy</td>
<td>Plasmacytic, 127</td>
<td>&lt;15% plasmablasts</td>
<td>39.7 mo</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mixed plasmablastic/plasmacytic, 35</td>
<td>20%-50% plasmablasts</td>
<td>16.1 mo</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>220</td>
<td>Bone marrow biopsies, smears, and imprints (myelotomy drill or Jamshidi needle)</td>
<td>Plasmablastic, 32</td>
<td>&gt;50% plasmablasts</td>
<td>9.8 mo</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Plasmablastic, 149</td>
<td>“Typical small plasma cells or ‘micro’ plasma cells”</td>
<td>41 mo</td>
<td>.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Plasmablastic, 71</td>
<td>“Cells with clear round nuclei usually with central single nucleoli and variable amount of basophilic cytoplasm and occasional perinuclear clear zone”</td>
<td>19 mo</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>82</td>
<td>NA</td>
<td>Plasmablastic, NA</td>
<td></td>
<td>30.5 mo</td>
<td>&lt;.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Plasmablastic-plasmacytic, NA</td>
<td></td>
<td>16.4 mo</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Plasmablastic, NA</td>
<td></td>
<td>4.6 mo</td>
<td></td>
</tr>
<tr>
<td>This work</td>
<td>100</td>
<td>Iliac crest aspirate (direct marrow smear)</td>
<td>Not plasmablastic, 85</td>
<td></td>
<td>35 mo</td>
<td>&lt;.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Plasmablastic, 15</td>
<td></td>
<td>10 mo</td>
<td></td>
</tr>
</tbody>
</table>

NA, not available.

vival for patients with plasmablastic myeloma and related this to higher tumor burden, higher serum creatinine concentration, and lower hemoglobin level.

Criteria for selection of morphological subtypes must be clear, specific, and reproducible for a morphological classification system to be successfully applied. Our system is similar to that proposed by Bayrd\textsuperscript{6} in 1948. He studied sternal bone marrow aspirates from 41 cases of multiple myeloma. Of these, ten (24\%) had grade 3 myeloma as defined by the presence of a significant number of very immature plasma cells. Survival for these patients was less than one year, compared to a survival of greater than two years for grade 1 and variable survival for grade 2.

Our system for identifying plasmablastic myeloma did not depend on characterization of plasma cells from biopsy material or on a subjective estimate of percentage of very atypical plasma cells; rather, it was based on enumeration, by differential count, of the percentage of clearly defined plasmablasts and immature, intermediate, and mature plasma cells in a routinely obtained direct bone marrow aspirate preparation. The system is simple and the groups are distinct (Table 3). There is no overlap with respect to percentages of plasma cell types required to fulfill the criteria defined in Table 2. Such a system can be applied to the majority of patients with myeloma. The initial results encourage us to determine whether we can establish reproducibility of this system in a number of other clinical centers.

In the current study, we were careful to exclude patients with smoldering multiple myeloma\textsuperscript{4} or monoclonal gammmopathy of undetermined significance.\textsuperscript{3} In other studies, the date of onset of symptoms\textsuperscript{7} or date of biopsy\textsuperscript{8} was used to determine survival, or the date was not specified. Patients in our study included only those about to begin chemotherapy for multiple myeloma, and survival was measured from the date chemotherapy was begun. Differences in percentage plasmablasts required to make a diagnosis of plasmablastic myeloma between ourselves and another group\textsuperscript{7} cannot be easily reconciled because the criteria defining plasmablasts are different. A cooperative study is needed to resolve these differences and to develop universally accepted criteria for recognizing plasmablastic myeloma.

We were unable to ascertain a difference in clinical stage among patients with plasmablastic or other morphology, even though this has been reported by others.\textsuperscript{8,9} However, in the plasmablastic type, there was a slightly increased frequency of renal insufficiency and hypercalcemia, which is similar to Bartl's experience.\textsuperscript{8} Both of these features have been observed to be the principal variables affecting survival in a multivariate regression analysis of 219 cases by Kyle and Elveback.\textsuperscript{10} In addition, plasmablastic myeloma patients had increased frequency of other features observed to affect survival—notably, urine monoclonal light chain excretion greater than 1 g/24 h\textsuperscript{11} and plasma cell labeling index greater than or equal to 1%.\textsuperscript{12} In this
series, only increased serum creatinine concentration and increased labeling index were significantly correlated with survival.

In conclusion, morphological classification of myeloma did recognize an unfavorable group with plasmablastic features and promises to be a significant predictor of survival in patients with multiple myeloma. More frequent renal insufficiency and higher plasma cell labeling index may contribute to the shortened survival observed in patients with plasmablastic myeloma. A cooperative study of the impact of morphological classification in myeloma is needed.

REFERENCES


Multiple myeloma: significance of plasmablastic subtype in morphological classification

PR Greipp, NM Raymond, RA Kyle and WM O'Fallon