Prognostic Relevance of Cellular Morphology in Multiple Myeloma

By Elke Fritz, Heinz Ludwig, and Michael Kundi

Morphological characteristics of tumor cells have been employed in the prognosis of lymphomas and solid tumors. This report documents an attempt to predict survival from the known cytologic heterogeneity in multiple myeloma. Myeloma cells in bone marrow smears from patients at diagnosis were evaluated by assigning them to morphologically defined categories. Cox’s multivariate regression model for censored survival data was used to generate optimal weights, which served as coefficients in two regression equations to estimate death risk from cellular morphology. Step-wise procedures excluded redundant parameters. "Myeloma morphology score" (MMS) discriminates significantly (p < 0.0001) among 3 stages, with median survival times of 42.5, 30.7, and 9.1 mo. For clinical routine application, "myeloma progression score" (MPS), the weight sum of the proportion of plasmablasts and the extent of bone marrow plasma cell infiltration, is suggested as a simple prognostic tool. Its discriminative power is very high (p < 10^-7). Median survival times of >71.5, 23.4, and 6.1 mo were found for good, moderate, and poor risk groups, respectively. However, staging is not confined to three subgroups, grouping is flexible, and pairs of data can be matched. This fact may prove to be valuable in designing prognosis-controlled clinical trials or theoretical studies on cellular differentiation. Preliminary results suggest changes in morphology due to disease progression and/or the effect of therapy on tumor kinetics. Most importantly, staging according to MPS or MMS may facilitate the adaption of therapy to the current state of the disease in patients with multiple myeloma.

THE PROGNOSTIC RELEVANCE of a morphological classification system has long been recognized in the clinical approach to lymphomas and solid tumors. Repeated histologic examinations of malignant tumor tissues have often revealed significant dedifferentiation and loss of specialized cellular functions during the progression of tumor growth. In multiple myeloma, advancement of the disease has been assumed to be associated with an increase in cytologic heterogeneity of myeloma cells, though up to now, only a few authors have attempted to systematically investigate the correlation between cellular morphology and survival. To our knowledge, the morphological classification of myeloma cells as a prognostic tool has not been examined as yet. The extent of bone marrow plasma cell infiltration, however, has been shown to correlate with survival. Therefore, it was our aim to investigate the relationship between morphological criteria of myeloma cells and the survival times of patients with multiple myeloma. We intended to express cellular differentiation quantitatively by means of assigning optimal weights to the various myeloma cell categories. An additional project was to ascertain whether the previously proposed prognostic relevance of the degree of bone marrow plasma cell infiltration could be supported by our data. Ultimately, we attempted to establish a simple prognostic measure that would optimally utilize both the quantitative and the qualitative information derived from bone marrow smears in multiple myeloma.

MATERIALS AND METHODS

Patients

The diagnosis of multiple myeloma was established according to standard criteria, requiring the presence of at least two of the following characteristics: (1) serum or urinary M-component, (2) bone marrow plasma cell infiltration >15%, and (3) osteolytic bone lesions. Between 1975 and 1982, a total of 108 patients with multiple myeloma were included in the study. Sixty-five presented with IgG, 22 with IgA, 1 with IgD, 17 with light chain, and 3 with nonsecretory myeloma. Forty-four patients were investigated twice. The resulting 152 differentiated bone marrow smears were divided into two groups: 85 smears from patients at the time of diagnosis prior to any form of treatment and 67 additional observations from patients after various periods of therapy. A separate group consisted of bone marrow smears from 12 patients with benign monoclonal gammopathy.

Bone Marrow

Bone marrow was aspirated by sternal or iliac puncture. Smears were prepared and routinely stained with May-Grünwald-Giemsa staining. Differential counts were made on 500 consecutive cells in each preparation under conditions that excluded concurrent knowledge of the patient’s history.

Morphology

Evaluation of the bone marrow cells consisted of assigning them to one of the following seven categories.

(A) Mature plasma cells were indistinguishable from normal plasma cells (Marschalko cells), i.e., ovaly shaped with a dense, eccentrically located nucleus, basophilic cytoplasm, and a perinuclear clear area.

(B) Cells with signs of asynchrony were characterized by a large nucleus of light density and often with several prominent nucleoli in

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a dark blue, mature cytoplasm, or a mature dense nucleus surrounded by sparse light blue cytoplasm.  

(C) Plasmablasts were distinguished by their centrally located large and immature nucleus, several nucleoli, and a relatively small, light blue rim of cytoplasm.  

(D) Lymphoplasmacytoid cells were similar in size to lymphoblasts, but showed distinct features of plasma cell differentiation, such as a perinuclear clear area, dark blue cytoplasm, or wheel-spoke pattern of chromatin.  

(E) The number of multinucleated myeloma cells, i.e., all myeloma cells containing more than one nucleus, was recorded in addition to assigning them individually to the respective differentiation class (A through C only).  

(F) Lymphoblasts were counted in order to examine the possible influence of a morphologically unidentified, hypothetical myeloma precursor cell population. However, the magnitude of plasma cell infiltration of the bone marrow was determined conventionally and did not include lymphoblasts.  

(G) Other bone marrow cells designated all remaining nucleated cells (Fig. 1A).  

Statistical Evaluation  
The amount of cells encountered in each category of bone marrow cells was expressed as relative frequency. For each of the six morphological variables, for the percentage bone marrow plasma cell infiltration, and the patient’s age the proportionality assumption was tested by means of the log-minus-log survival function. Cox’s regression model for censored survival data was applied using BMDP-P2L program on CICS of an IBM 43 41 computer at the Institute of Medical Computer Science, University of Vienna. Two sets of data from patients at diagnosis were entered into the model without prior transformation. One consisted of the relative frequencies in the myeloma cell categories, percentage bone marrow plasma cell infiltration, and age, and the second was confined to morphology, i.e., the frequencies of the various cellular variables were standardized for the total of observed myeloma cells in each patient. Step-up procedures were applied using Peduzzi-Hardy-Holford statistics for preliminary elimination and continuing with the maximum partial likelihood test. Survival time was measured from the time of the bone marrow puncture in question to death or observational loss. The two resulting sets of coefficients regarding significant variables were standardized for mature myeloma cells and total of myeloma cells to yield the regression equation for “myeloma morphology score” (MMS) and “myeloma progression score” (MPS), respectively.  

After calculation of MMS and MPS for each case, bivariate scatter plots, displaying the respective scores against survival times, were generated by means of BMDP-P6D program. No obvious clustering was noticed, confirming the assumption of MMS and MPS as a continuous nonlinear measure. Stratification into three stages was performed by setting cutpoints arbitrarily at the 33.3rd and 66.7th percentile. Plots of cumulative survival were derived by the method of Kaplan and Meier. For comparisons among the three groups, both the Mantel-Cox and the Breslow test, as contained in BMDP-P1L program, were used. The Breslow test gives greater weight to early observations and is less sensitive to late events that occur when few patients on the study remain alive.  

RESULTS  
Log-minus-log plots confirmed the linear correlation between each myeloma cell type and survival, a presupposition for the application of Cox’s multivariate regression model (data not shown).

The coefficients derived by Cox’s regression analysis, which assigns optimal weights to the morphological categories of myeloma cells, are shown in Table 1A. Step-wise procedures proved that each of the listed cell types contributed significantly ($p < 0.05$) to the probability of survival. Because the coefficients derived for mature plasma cells and asynchronous myeloma cells (designated in Fig. 1 as A and B, respectively) were identical, both cell types were subsumed under the new category “plasmacytes.” “Myeloma morphology score” (MMS) is defined as the sum of all parameters multiplied by their appropriate weights. Before being entered into the equation, all frequencies are divided by the total frequency of lymphoid-myelomatous cells in the smear of the specific patient. Therefore, MMS refers strictly to morphology and is independent of the magnitude of bone marrow plasma cell infiltration. Figure 2 demonstrates the discriminative power of MMS after division of the sample into three groups of approximately equal size. The significant result is of high theoretical interest, because it confirms the expected association between purely morphological cellular characteristics and survival in multiple myeloma.  

Figure 3 shows the highly significant prognostic value of bone marrow plasma cell infiltration as determined by cytologic differentiation of sternal or iliac puncture smears. In an attempt to combine the predictive power of morphological evaluation with the prognostic value of the extent of bone marrow plasma cell infiltration, the actual frequencies of the various myeloma cell types—which mirror both the qualitative and the quantitative aspect—were entered into Cox’s model. Input also included the total amount of myeloma cells in the bone marrow smear, because we suspected high correlations between the percentage plasma cells and the size of some morphologically defined cell compartments. In fact, a step-wise procedure excluded as redundant all parameters except the frequency of plasmablasts and the extent of bone marrow plasma cell infiltration. Standardization for the latter resulted in the regression equation defined as
Fig. 2. Cumulative survival of 85 patients categorized by staging according to myeloma morphology score: Stage I = MMS < 1.015, stage II = MMS 1.015–1.120, stage III = MMS > 1.120. The respective median survival times are 42.5, 30.7, and 9.1 mo. Subgroups are approximately equal in size (n = 29, 28, and 28). The differences are highly significant (Breslow: p < 0.0002; Mantel-Cox: p < 0.0001).

Fig. 3. Cumulative survival of 85 patients categorized by staging according to percentage bone marrow plasma cell infiltration: Stage I = <15%, stage II = 15%–40%, stage III = >40%. The respective median survival times are 50.2, 42.5, and 5.7 mo, the number of cases 34, 26, and 25. The differences are highly significant (Breslow: p < 10^{-4}; Mantel-Cox: p < 10^{-7}).
“myeloma progression score” (MPS), which is stated in Table 1B. Figure 4 shows the highly significant capacity of MPS to differentiate among good, moderate, and poor risk in multiple myeloma.

Figure 5A and Fig. 6 demonstrate cumulative survival of our patient population according to the risk criteria suggested by the Myeloma Task Force, namely, levels of hemoglobin, blood urea nitrogen (BUN), and serum calcium, and to the staging system by Durie and Salmon, respectively. The discriminative power of our proposed predictive measures MPS (Fig. 4) proved to be at least equal to the discrimination obtained by these well-established prognostic classification systems.

The distribution of our patients among the various staging systems is shown in Table 2. In Table 2A, no correlation between either MMS or MPS and the risk criteria of the Myeloma Task Force was found, while Table 2B demonstrates the significant correlation between both of our measures and Durie and Salmon’s staging system. Correlation between prognosis by means of the Myeloma Task Force criteria and staging according to Durie and Salmon amounted to $r_{cc} = 0.268 (p < 0.05; data not shown). Because of the substantial differences in the size of subgroups, the correlation coefficients obtainable at maximum correspondence would only be 0.646, 0.701, and 0.552 for 2A, 2B, and the latter comparison, respectively.

Figure 5B is an example of the possibility of employing MPS for a more subtle discrimination within a large-sized group obtained by another staging system. Patients classified as good risk according to the criteria of the Myeloma Task Force can be assigned to subgroups with significantly different survival expectancies.

Grouping according to paraprotein classes (IgG, IgA, and light chain myeloma) showed no significant differences for either MMS or MPS or survival. The seven cases of lambda light chain myeloma suggested shorter survival and higher scores without reaching statistical significance at the 5% limit (data not shown).

A preliminary estimation of the prognostic relevance of changes in cellular morphology during the course of the disease under treatment is presented in Fig. 7. Differences were calculated between MMS at diagnosis or first encounter and MMS at the time of the repeated bone marrow examination. Observations with a decrease in MMS were compared to those with
Table 2. Correlations of MMS and MPS With Other Staging Systems

<table>
<thead>
<tr>
<th></th>
<th>MMS I</th>
<th>MMS II</th>
<th>MMS III</th>
<th>Total</th>
<th>MPS I</th>
<th>MPS II</th>
<th>MPS III</th>
<th>Total</th>
</tr>
</thead>
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<tr>
<td>(A) Comparison of MMS and MPS to prognostic criteria of the Myeloma Task Force</td>
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<td></td>
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<td></td>
<td></td>
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<tr>
<td>Good risk</td>
<td>24</td>
<td>20</td>
<td>18</td>
<td>62</td>
<td>24</td>
<td>20</td>
<td>18</td>
<td>62</td>
</tr>
<tr>
<td>Poor risk</td>
<td>5</td>
<td>8</td>
<td>10</td>
<td>23</td>
<td>4</td>
<td>8</td>
<td>11</td>
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<tr>
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<td>29</td>
<td>28</td>
<td>28</td>
<td>85</td>
<td>28</td>
<td>28</td>
<td>29</td>
<td>85</td>
</tr>
<tr>
<td>$r_{cc}^a$</td>
<td>0.169</td>
<td>not significant</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

| (B) Comparison of MMS and MPS to staging according to Durie and Salmon (D&S) |
| D & S I        | 8     | 6      | 2       | 16    | 10    | 5      | 1       | 16    |
| D & S II       | 15    | 9      | 6       | 30    | 13    | 9      | 8       | 30    |
| D & S III      | 6     | 13     | 20      | 39    | 5     | 14     | 20      | 39    |
| Total          | 29    | 28     | 28      | 85    | 28    | 28     | 29      | 85    |
| $r_{cc}^a$     | 0.389 | $p < 0.005$ |

$^a$Correlation: Pearson's contingency coefficient.

DISCUSSION

The clinical necessity to optimize the therapeutic strategies presently available for the treatment of multiple myeloma has led to increased emphasis on prognosis and the evaluation of death risk. Several clinical parameters have been found to correlate with survival. Parameters related to kidney function, hemoglobin, the level of serum calcium, and the performance status especially tend to deteriorate with the progression of the disease and, therefore, have served as prognostic factors. Multimorbidity in the usually elderly patients, however, may adulterate prognosis by introducing changes in the significant parameters through coexistent diseases. The identification and establishment of tumor-specific prognostic factors other than serum or urinary M-component could help to eliminate or diminish the influence of these artefacts.

By means of a multivariate regression analysis of survival data, we demonstrated the highly significant...
prognostic value of a morphological classification of bone marrow cells in multiple myeloma. This seems to be of particular interest, because it implies comparability between cellular growth patterns during the progression of multiple myeloma and the dedifferentiation processes known to occur in the course of other tumors. Myeloma morphology score (MMS), a sum of the weight frequencies of various relevant myeloma cell types, is a measure of tumor cell maturation exclusively, as it is independent of the magnitude of bone marrow plasma cell infiltration. Its power to discriminate between patient groups with different survival expectancies confirms the relevance of cytologic criteria in prognosis.

It is interesting to note that the coefficients for mature and for asynchronous plasma cells were identical. This result does not necessarily contradict the reports of increased asynchrony in progressive myeloma, rather, it seems to imply very similar patterns of cellular reproduction in mature and in asynchronous myeloma cells. The evaluation procedure involved in generating MMS might appear to be work-intensive, but the body of cytologic information contained in this single score makes it a promising tool, particularly for investigational purposes, such as a more detailed study of tumor kinetics.

Myeloma progression score (MPS), on the other hand, is a very simple prognostic measure with the advantage for routine application of including only two parameters, namely, the proportion of plasmablasts and the extent of bone marrow plasma cell infiltration. As a continuous measure, it allows the placing of a single case within the spectrum of the entire patient group. MPS does not imply preexisting stages; rather, it permits the division of a group of patients—depending on its size—into two to four or more subgroups, thus avoiding the disadvantage of a markedly unequal distribution among preexisting stages. Even if absolute survival times differ at various treatment centers and might change with further improvement of therapy, at least at the time of diagnosis, the relative degree of cellular differentiation remains comparable, and MPS can be assumed to rank survival expectancy reliably.

We refrained from specifying a regression equation that could be used to convert the MPS of an individual patient into his expected survival time. Survival in individual cases varies considerably, and we prefer to state the particular risk of a patient in terms of the percentiles derived from the standard patient collective analyzed in this article. Thus, the degree of risk for the individual patient can be interpreted within the context of accumulated personal clinical experience.

The estimation of death risk plays an important role in the adaption of the therapy to the current state of the disease. Like other staging systems, MPS can be used to identify good, moderate, and poor risk in patients with multiple myeloma. However, the continuity of the quantitative measure MPS not only provides greater flexibility in staging, it also can be employed to reduce the large number of patients usually necessary in clinical trials that test the benefit of new treatments. Patients with comparable MPS can be paired and randomly assigned to alternative therapy groups. A listing of selected percentiles (Table 3) offers options of cutpoints for matching and grouping according to the value of MPS.

Apparently, cellular morphology adds a dimension to prognosis that is largely independent of the clinical parameters included in the risk criteria as defined by the Myeloma Task Force. Lack of correlation between good versus poor risk and our prognostic measures was found not only for cutpoints at the 33.3rd and 66.7th percentile (Table 2A), but also for dichotomous staging by placing the cutpoint either at the 50th or the 73rd percentile. The latter resulted in distribution among the two subgroups that was equivalent to the one achieved by the Myeloma Task Force risk criteria (data not shown). The correlation observed between the distribution according to the Myeloma Task Force and Durie and Salmon’s staging systems might be accounted for by the overlap of the parameters of hemoglobin and serum calcium in both systems. The additional tumor-specific information on paraprotein and the relatively stable criterion of bone lesions contained in the staging system by Durie and Salmon might, on the other hand, coincide with morphological dedifferentiation of the myeloma cells. This possible explanation of the correlation found between staging according to Durie and Salmon and our proposed prognoses is further supported by observations on the mode of bone infiltration by myeloma cells and the degree of plasma cell maturation.

Table 3. MPS: Selected Percentiles in the Standard Patient Population

<table>
<thead>
<tr>
<th>Percentile</th>
<th>MPS</th>
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<tbody>
<tr>
<td>10</td>
<td>0.0375</td>
</tr>
<tr>
<td>20</td>
<td>0.0704</td>
</tr>
<tr>
<td>25</td>
<td>0.0803</td>
</tr>
<tr>
<td>30</td>
<td>0.0927</td>
</tr>
<tr>
<td>33.3</td>
<td>0.1040</td>
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<tr>
<td>40</td>
<td>0.1616</td>
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<tr>
<td>50</td>
<td>0.2320</td>
</tr>
<tr>
<td>60</td>
<td>0.3514</td>
</tr>
<tr>
<td>66.7</td>
<td>0.4260</td>
</tr>
<tr>
<td>70</td>
<td>0.4764</td>
</tr>
<tr>
<td>75</td>
<td>0.5653</td>
</tr>
<tr>
<td>80</td>
<td>0.6702</td>
</tr>
<tr>
<td>90</td>
<td>1.0635</td>
</tr>
</tbody>
</table>
In a number of patients, especially in those with advanced disease, progressive dedifferentiation of myeloma cells seems to be associated with a decrease or loss of paraprotein synthesis.\textsuperscript{7} This process has also been observed in established cell lines, where immunoglobulin production became restricted to free light chains.\textsuperscript{28} Therefore, in light chain myeloma, poorly differentiated tumor cells and, consequently, a high MMS could be expected. Our data supported this hypothesis only for the group of patients with lambda light chain myeloma, but the number of cases was too small to provide a sound basis for statistical evaluation.

The significant difference between malignant myeloma and benign monoclonal gammapathy detected especially for MPS might prove to be of clinical value. MPS below a certain limit could possibly serve as an additional criterion in differential diagnosis between benign monoclonal gammapathy and multiple myeloma and might facilitate decision making concerning the initiation of chemotherapy. For that purpose, our results must be confirmed by a prospective investigation.

The changes of MMS observed upon repeated morphological evaluation led to an interesting speculation: With the advancement of multiple myeloma, a progressive rise of MMS can be expected as a sign of cellular dedifferentiation during the course of the disease. Successful treatment with cytostatic drugs might cause a selective elimination of less differentiated plasma cell clones and, consequently, the retardation of malignant growth; however, this transient effect on morphology should not be observable in patients resistant to chemotherapy. In fact, comparison of survival in patients who showed an increase in MMS during the interval to those whose MMS decreased, demonstrated an association between decreases in MMS and longer survival times. However, our 44 observations after various periods of therapy were too sporadic to provide a basis for definite conclusions. A prospective investigation under standardized conditions, such as predetermined intervals, seems to be obligatory.

At present we are in the process of verifying the value of MPS as a prognostic tool on an independent large sample of bone marrow smears that were prepared from patients with multiple myeloma at the time of diagnosis at another treatment center. If reliability and validity of this predictive measure can be confirmed, prognosis in patients with multiple myeloma could easily be monitored and treatment could be adjusted accordingly, e.g., the poor prognosis of a rapid decrease of the tumor load\textsuperscript{3} might be manifested by a predominance of blastoid myeloma cells, and the resulting relatively high MMS would call for maintenance therapy during remission. Ultimately, we intend to make use of all prognostic indicators presently available by combining morphological evaluation with other clinical parameters of known prognostic value. This way, we hope to achieve survival predictions that are sufficiently precise for a clinical application to individual patients with multiple myeloma.

**APPENDIX: CALCULATION OF MMS and MPS**

The following example demonstrates the steps of prognostic evaluation of a typical bone marrow smear from a patient with multiple myeloma at the time of diagnosis.

(1) Differential count of the bone marrow smear yielded

- plasmacytes 98
- plasmablasts 26
- lymphoplasmacytoid cells 9

**MMS**

\[
\text{MMS} = \frac{98}{158} 0.620 + \frac{26}{158} 0.165 + \frac{9}{158} 0.057 = 0.387
\]

(2) Calculation of myeloma morphology score (MMS)

(A) Standardized frequencies are determined by dividing the number of cells in each category by the number of lymphoid-myelomatous cells.

- plasmacytes 98/158 0.620
- plasmablasts 26/158 0.165
- lymphoplasmacytoid cells 9/158 0.057
- lymphoblasts 25/158 0.158

(B) The calculated frequencies are substituted into the formula.

\[
\text{MMS} = 0.620 + (0.165 \times 1.904) + (0.057 \times 1.095) + (0.158 \times 0.772) - 1.18 - \text{MMS}
\]

(C) If staging is desired, the MMS places the patient at the upper limit of stage II.

(3) Calculation of myeloma progression score (MPS)

(A) Relative frequencies are calculated by dividing the absolute number by the total number of evaluated bone marrow cells.

- plasmablasts 26/500 0.052
- myeloma cell infiltration 133/500 0.266

(B) The calculated frequencies are substituted into the formula.

\[
\text{MPS} = (0.052 \times 2.327) + 0.266 - 0.387 = \text{MPS}
\]

(C) The percentile is estimated roughly or by graphical interpolation.

\[
0.351 < 0.387 < 0.426
\]

60th percentile 66.7th percentile

The patient is assigned the 64th percentile of the standard patient population, indicating that his survival expectancy is exceeded by 63% of all patients with multiple myeloma, whereas in 35% of the cases, prognosis is poorer. Staging places the patient at the upper limit of the intermediate stage II.

**REFERENCES**

Prognostic relevance of cellular morphology in multiple myeloma

E Fritz, H Ludwig and M Kundi