Clinical, Morphological, and Cell Kinetic Differences Among Multiple Myeloma, Monoclonal Gammopathy of Undetermined Significance, and Smoldering Multiple Myeloma

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We reviewed the clinical and morphological findings in 43 cases of monoclonal gammopathy of undetermined significance (MGUS), 9 of smoldering multiple myeloma (SMM), and 23 of overt multiple myeloma (MM). In all cases, the patients' physicians had requested a bone marrow examination because of the possibility of MM. In all 75 cases, ^3H-thymidine labeling indices were performed. The plasma cell labeling index correctly classified 62 of the 75 cases (83%). A linear discriminant function combining the labeling index and percentage of plasma cells improved the accuracy to 92% (69/75), or to 96% (71/75) if patients in whom MM developed within 6 mo were considered to have MM. The labeling index was most critical for the differential diagnosis of MM from SMM (p<0.001). Serum or urine M-protein level, percentage of plasma cells or lymphocytes in the bone marrow, and plasma cell grade, asynchrony, and nucleolar size failed to discriminate the group with SMM from the group with MM. In patients with MGUS or SMM, a plasma cell labeling index greater than 0.4% warned of impending MM. The plasma cell labeling index is a reliable diagnostic test when applied in cases of monoclonal gammopathy, especially when differentiation from MM is difficult using standard clinical criteria.

Definitions

At the time of study, patients with MGUS had levels of serum monoclonal protein less than 3 g/dl or less than 10% plasma cells in the bone marrow and no other evidence of MM (that is, no bone lesions, anemia, or renal insufficiency).

Patients with SMM had more than 3 g/dl of monoclonal protein in the serum and more than 10% plasma cells in the bone marrow at diagnosis. None had clinical or other laboratory evidence of overt symptomatic MM, and none had been treated.

Patients with MM had clinical findings consistent with the diagnosis and had more than 10% plasma cells in the bone marrow plus osteoporosis or lytic lesions, together with the finding of a monoclonal protein in the serum or urine (or both). Patients without bone lesions had more than 30% plasma cells in the bone marrow, and patients with less than 10% plasma cells in the bone marrow had lytic bone lesions or progressive osteoporosis with fractures. Ancillary features included bone pain, anemia, and elevated calcium and creatinine levels.

Exclusions

Patients with solitary plasmacytoma (bone or extramedullary), IgM monoclonal gammopathy, macroglobulinemia of Waldenström, and primary amyloidosis (AL) were excluded.

Labeling Indices

High-speed scintillation autoradiography was utilized with the following five modifications. (1) Hanks' balanced salt solution was buffered with bicarbonate to a pH of 7.2–7.3. (2) Bovine serum albumin (BSA) was substituted for dextran in the bone marrow sedimentation procedure (final concentration 3 g/dl). (3) Fetal calf serum (FCS) was not used. (4) Tritiated thymidine (high specific activity 40–60 Ci/mmol) was added during the sedimentation of the marrow (final concentration 5 μCi/ml of marrow) and incubated for 1 hr. (5) A 36-hr exposure period was routinely used, and a temperature of –70°C in a calcium chloride dry box was used to achieve maximal labeling, as judged by dense accumulation of grains over erythroid nuclei.

The plasma cell labeling index was determined by counting 500 cells and determining the percentage with more than 5 grains over the nucleus. Lymphocyte labeling indices were performed in a similar manner. Background contained less than 1 grain per cell area. To measure the variability of the labeling index procedure, we
Differences among MM, MGUS, and SMM

Compared two separate labeling index determinations on 20 patients. Radioautographs were coded prior to reading by one observer. The coefficient of variation for each pair was calculated, and the average coefficient of variation (CV) was determined. The CV was 0.37 for labeling indices ±1% (range 0–1.42) and was 0.18 for indices >1% (range 0–0.4) (Fig. 1). The low variability of the labeling indices allowed meaningful comparison of results among different patients.

**Morphology Studies**

Wright-stained direct marrow preparations were examined using a 100× oil immersion objective and a calibrated micrometer ocular (1 division = 1.02 μm). The percentage of plasma cells was determined on a count of 500 bone marrow cells. Twenty sequentially identified plasma cells from each bone marrow sample were subjected to morphological study. Values were recorded as the mean.

Nucleolar size was expressed as the diameter. When irregular or oval nucleoli were found, the longest and shortest diameters were averaged. When multiple nucleoli were present, one was usually much larger than the others, and the larger one was used. If only multiple small nucleoli were present, the diameter was expressed as the sum of the small diameters. Nucleolar size was categorized as follows: 0 = none; 1 = small (<1 μm); 2 = intermediate (1–3 μm); and 3 = large (>3 μm).

Nuclear size was expressed as the nuclear diameter. When oval, nuclei were recorded as the average of the longest and shortest diameters. Nuclear size was further categorized as follows: small (<8 μm), intermediate (8–10 μm), and large (>10 μm).

Plasma cell grade was determined using data from nucleolar and nuclear size and appearance of chromatin: grade 0 = mature, no atypia; grade 1 = mature, mild atypia, small nucleolus and nucleus, and condensed chromatin; grade 2 = intermediate (neither grade 0, 1, or 3); grade 3 = immature, large nucleolus and nucleus, and dispersed chromatin (at least two of the above); grade 4 = blastic, large nucleolus and nucleus and dispersed chromatin (all of the above). Rarely, a cell could not be fitted into this scheme.

Plasma cell asynchrony (nuclear cytoplasmic maturation asynchrony) was determined using criteria developed from the methods of Bernier and Graham. Nuclei were graded 1, 2, or 3, as done for plasma cell grade, except for blastic morphology, which was graded 3 rather than 4 for determination of asynchrony, and grade 0, which was incorporated into grade 1. Cytoplasm was graded as follows: grade 1 = mature, light blue, abundant with a prominent hof, grade 2 = intermediate (neither grade 1 nor 3); and grade 3 = immature, dark blue, less abundant, lack of a prominent hof. Asynchrony was determined by the equation: asynchrony = nuclear grade minus cytoplasmic grade. Possible scores were 0, 1, and 2 (negative values were recorded as 0).

**Statistical Analysis**

Analyses (Kruskal-Wallis test) included comparisons of the three groups by serum and urine M-protein levels, percentages of plasma cells and lymphocytes in bone marrow, nucleolar size, plasma cell grade and asynchrony, and plasma cell and lymphocyte labeling indices. Correlation between the labeling index and other variables was assessed using Spearman's correlation coefficient. Linear discriminant analysis identified the two most reliable variables—plasma cell labeling and percentage of plasma cells—and was used to derive a discriminant function that separated myeloma (MM) from nonmyeloma (MGUS and SMM) cases (Fig. 2).

**RESULTS**

**Patient Characteristics and Outcome**

**MGUS.** Forty-three patients (20 males and 23 females; with a median age of 66 yr) had MGUS. Intact serum monoclonal proteins were found in 40: IgG in 31 and IgA in 9. Of the 40, 21 had κ and 19 had λ chains. Two patients had bicalonal gammopathy; one had IgGκ plus IgAλ and the other had IgGκ plus IgMλ. One patient had free light chain in the serum and no intact monoclonal immunoglobulin (Bence Jones proteinemia). This patient had 1.0 g/24 hr of light chain in the urine and 8% plasma cells in the marrow and has had no evidence of overt MM during 42 mo of follow-up. Urinary Bence Jones protein was demonstrated in 20 patients; 9 had κ and 11 had λ chains.

Symptomatic MM developed in 5 patients initially recognized as having MGUS; in 2 of the 5, the MM developed within 6 mo. Initially, one had 3% plasma

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**Fig. 1.** Plasma cell labeling index (LI). Mean coefficient of variation for paired observations by one observer (diagonal line represents ideal reproducibility).

**Fig. 2.** Linear discriminant function analysis between multiple myeloma (MM) and nonmyeloma (MGUS and SMM), using plasma cell labeling index and percentage of marrow plasma cells. The line correctly classified 92% (69/75), or 95% (71/75) if the patients in whom MM developed in less than 6 mo are considered patients with MM.
cells in the marrow and 3.9 g of monoclonal protein in the
serum. At diagnosis of MM 6 mo later, this patient had
lytic bone lesions, 4.3 g/dl of monoclonal protein in
the serum, and 7% plasma cells. The other patient had
1.0 g/dl of monoclonal protein in the serum both
initially and 3 mo later at the diagnosis of MM, but
progressive osteoporosis and painful compression frac-
tures developed, necessitating treatment. Both of these
patients probably had MM from the outset. The 3
other patients developed symptomatic MM after
remaining stable for 8–31 mo. All other patients with
MGUS remained free of symptoms of MM and received
no treatment for 1–5 yr (median 26 mo). Two
died of unrelated causes.

SMM. Nine patients had SMM. All nine were
asymptomatic, and none had received treatment ini-
tially. Six have been followed from 5 to 16 yr without
evidence of MM and have been reported on previous-
ly.4 Another patient has been stable for 36 mo.
Initially, this patient had 3.8 g/dl of IgAα monoclonal
protein in the serum and 21% plasma cells in the
marrow.

Symptomatic MM developed in two patients, one
after 2 mo and the other after 33 mo. The first patient
had 3.0 g/dl of monoclonal protein in the serum and
23% plasma cells in the bone marrow at the outset, but
had no lytic lesions or anemia and was asymptomatic.
Two months later, symptomatic MM was recognized
when lytic lesions developed and the serum M-protein
level increased to 3.7 g/dl. This patient probably had
active MM at the outset. The second patient appeared
to undergo a transition. Initially, he had 3.6 g/dl of
IgGα monoclonal protein in the serum and 24% plasma
cells in the bone marrow. Levels of serum monoclonal
protein and marrow plasma cells remained stable for
33 mo, after which he developed symptomatic MM
with lytic bone lesions, 4.9 g/dl of monoclonal protein
in the serum, and 36% plasma cells in the bone marrow
and required treatment.

MM. Twenty-three patients (14 males and 9
females with a median age of 61 yr) had symptomatic
MM initially. All were treated just after diagnosis.
IgG monoclonal proteins were found in the serum of
13, IgA in 5, and IgD in 1; 8 had κ and 11 had λ chains.
Four patients had free light chains (all λ) in the serum
and no heavy chains. Urine monoclonal light chains
were present in 15 (5 κ and 10 λ).

Comparisons and Correlations Among Groups

The plasma cell labeling index was tenfold higher in
MM than in MGUS (p<0.0001). A significant differ-
ence between MM and MGUS was observed for
percentage of plasma cells (p<0.0001). Other
observed differences included level of serum mono-
clonal protein (p<0.05) and level of urine monoclonal
protein (p<0.001) (Table 1). Plasma cell atypia was
greater in MM than in MGUS, and the differences
were statistically significant for nucleolar size
(p<0.0001), grade (p<0.0001), and asynchrony
(p<0.01). The percentage of lymphocytes in the mar-
row was lower in MM than in MGUS (p<0.01). There
was no significant difference in the lymphocyte label-
ing index between MM and MGUS.

Except for the plasma cell labeling index, the differ-
ences between MM and SMM were less impressive
(Table 1). The plasma cell labeling index was tenfold
higher in MM than in SMM and was the most reliable
discriminant among the variables tested (p<0.001).
Levels of urine monoclonal protein, though different
(0.2 and 2.9 g/24 hr), did not reach statistical signifi-
cance, and levels of serum monoclonal protein and
nucleolar size, grade, and asynchrony were not signifi-
cantly different. The percentage of plasma cells was
lower in SMM than in MM (p<0.05). The number of
marrow lymphocytes was greater in SMM than in
MM (p<0.05). There was no significant difference in
the lymphocyte labeling index. When a plasma cell
labeling index of greater than 0.4% was used to sepa-
rate MM from MGUS and SMM, 83% were correctly
classified. A linear discriminant function (Fig. 2)
combining plasma cell labeling index and percentage
of marrow plasma cells improved the accuracy to 92% or
95% if patients in whom MM developed in less than
6 mo were considered to have MM. Addition of other
discriminant variables did not improve the differen-
tiation between MM and non-MM cases.

The same variables in Table 1 are shown in Fig. 3,
but their distributions are given so that differences
among the three groups can be further analyzed.
Levels of serum monoclonal protein in MM (mean 3.2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MM (23 Patients)</th>
<th>MGUS (43 Patients)</th>
<th>SMM (9 Patients)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum M-protein (g/dl)</td>
<td>3.2</td>
<td>2.2§</td>
<td>3.4</td>
</tr>
<tr>
<td>Urine M-protein (g/24 hr)</td>
<td>2.9</td>
<td>0.3†</td>
<td>0.2</td>
</tr>
<tr>
<td>Marrow plasma cells (%)</td>
<td>49</td>
<td>5*</td>
<td>17§</td>
</tr>
<tr>
<td>Marrow lymphocytes (%)</td>
<td>10</td>
<td>15‡</td>
<td>21§</td>
</tr>
<tr>
<td>Nucleolar size (μm)</td>
<td>1.6</td>
<td>1.0*</td>
<td>1.3</td>
</tr>
<tr>
<td>Grade</td>
<td>1.6</td>
<td>1.1*</td>
<td>1.3</td>
</tr>
<tr>
<td>Asynchrony</td>
<td>0.5</td>
<td>0.3‡</td>
<td>0.5</td>
</tr>
<tr>
<td>Plasma cell labeling index (%)</td>
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<td>0.1*</td>
<td>0.1†</td>
</tr>
<tr>
<td>Lymphocyte labeling index (%)</td>
<td>0.6</td>
<td>0.1</td>
<td>1.0</td>
</tr>
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</table>

Significant difference from MM value: (Kruskal-Wallis test; chi-square
approximation) *p < 0.0001; †p < 0.001; §p < 0.01; §p < 0.05.
Fig. 3. Distribution and mean values (horizontal bars) for clinical, morphological, and cell kinetic parameters in monoclonal gammopathy of undetermined significance (MGUS), smoldering multiple myeloma (SMM), and multiple myeloma (MM). Open circle indicates patients initially classified as having MGUS or SMM who developed overt MM within 6 mo.

DIFFERENCES AMONG MM, MGUS, AND SMM

levels (g/dl) were similar to those in SMM (mean 3.4 g/dl) and were higher than those in MGUS (mean 2.2 g/dl). Nine patients with MGUS had levels greater than 3 g/dl, but were classified by definition as having MGUS because they had less than 10% plasma cells in the marrow and thus did not fulfill our criteria for SMM. Only 7 of 23 patients with MM had more than 4 g/dl of serum monoclonal protein, in contrast to no patients with MGUS or SMM.

Urine monoclonal light chain was observed in 15 of 22 patients with MM tested; similarly, 14 of 37 patients with MGUS and 6 of 9 patients with SMM tested had monoclonal light chain detected in the urine. Mean levels were higher in MM (2.9 g/24 hr) than in SMM (0.2 g/24 hr) and MGUS (0.3 g/24 hr). However, 12 of 22 patients with MM tested had low mean levels of monoclonal urine protein (0–0.5 g/24 hr). Twenty-two of 37 patients with MGUS had no monoclonal light chain in the urine, but levels of 0.1–0.3 g/24 hr were found in 13 of 37 tested. In SMM, 3 of 9 had no monoclonal protein in the urine, but the other 6 had monoclonal light chains and levels of 0.1–0.5 g/24 hr. Of the 23 patients with MM, 6 had no detectable monoclonal light chain in the urine and 10 had high levels (>0.5 g/24 hr). One patient failed to fulfill the criteria for MM or SMM and was classified as having MGUS. She had 10.9 g/24 hr of monoclonal light chain in the urine, but less than 10% plasma cells in the bone marrow on multiple occasions, and has not developed MM during 42 mo of follow-up.

The mean percentage of plasma cells in the marrow was greater in MM (49%) than in SMM (17%) and MGUS (5%). Significant overlap was present. More than 5% plasma cells were found in 13 of 43 patients with MGUS. Two patients with MGUS, despite having less than 3 g/dl of monoclonal protein in the serum and less than 0.5 g/24 hr of monoclonal light chain in the urine, had more than 20% plasma cells in the bone marrow. These two patients met the criteria for MGUS but might have been classified as having SMM because of the high percentage of plasma cells in the bone marrow. Five percent or less plasma cells in the marrow were found in only 3 of 23 patients with overt MM. These patients had lytic bone lesions, osteoporosis, and compression fractures, and a progressive
course that clearly distinguished them from patients with MGUS or MM. Three of nine patients with SMM had more than 20% plasma cells in the bone marrow. We have seen patients with more than 50% plasma cells on repeated bone marrow samples who remained stable for years without treatment. Thus, overlap in the percentage of plasma cells among MM, SMM, and MGUS groups makes it difficult to differentiate these groups on this basis.

The mean percentage of lymphocytes in the marrow was lower in MM (10%) than in SMM (15%) and MGUS (21%). Although possibly participating in the plasma cell proliferative process, marrow lymphocytes were considered separate from plasma cells for this analysis, since they were not distinguishable from normal lymphocytes by standard morphological technique.

Mean nucleolar size was significantly greater ($p<0.0001$) in MM (1.6 µm) than in MGUS (1.0 µm). There was considerable overlap, but very large nucleolar size was more often seen in patients with MM than in patients with MGUS: 8 of 23 patients with MM had nucleolar size greater than 1.6 µm in diameter, compared to only 1 of 43 with MGUS. Small nucleolar size was seen more often in patients with MGUS than in patients with MM: 8 of 43 patients with MGUS had nucleolar size less than 0.7 µm compared to 0 of 23 patients with MM. A distinction between SMM and MM was impossible to make on the basis of nucleolar size, grade, and asynchrony.

Mean plasma cell labeling index was 1.0% in MM and 0.1% in MGUS and in SMM. Three patients, however (two with MGUS and one with SMM), had overt MM within 6 mo (see Fig. 3). Only the plasma cell labeling index (0.6%, 0.6%, and 0.8%, respectively) distinguished these three patients from others with MGUS and SMM who remained stable. The elevated labeling index alerted us to the possibility of MM; such cases should be followed closely. The serum or urine monoclonal protein level, percentage of marrow plasma cells, plasma cell nucleolar size, grade, and asynchrony, and lymphocyte labeling index of these three patients were not different from those of patients with MGUS and SMM (see Fig. 3). Interestingly, the percentage of marrow lymphocytes appeared to be lower in these three patients, as seen in some patients with MM.

Of the 49 patients with MGUS and SMM who remained stable for at least 6 mo, 30 (61%) had plasma cell labeling indices of 0%. In the remaining 19, the plasma cell labeling indices were very low (all ≤0.4%). However, a labeling index of 0% was found in only 2 of 23 patients with MM.

When data on all 75 patients were analyzed, there was a correlation between plasma cell labeling index and other variables: urine monoclonal protein level ($r = -0.27, p<0.05$), percentage of marrow plasma cells ($r = 0.50, p<0.0001$), percentage of marrow lymphocytes ($r = -0.33, p<0.05$), and plasma cell nucleolar size ($r = -0.47, p<0.0001$), grade ($r = 0.50, p<0.0001$), and asynchrony ($r = 0.35, p<0.05$). Within the group with MM, there were insufficient patients to identify a relationship between the labeling index and these variables.

**DISCUSSION**

Increasingly frequent recognition of patients with MGUS and SMM has produced an increased need for precision in distinguishing them from patients with overt MM. Treatment of asymptomatic patients should be avoided. Before initiating chemotherapy, one must consider the morbidity and expense of alkylator therapy, the possibility of producing drug resistance, and the potential for the development of acute leukemia. In our patients with MM, the incidence of acute leukemia after alkylator therapy was 1.9% (actuarial 2.8% at 5 yr, 10.1% at 10 yr).

Criteria for the diagnosis of MM have not satisfactorily resolved this problem; for instance, treatment may be safely withheld in certain patients satisfying accepted criteria for the diagnosis of MM. We have described 6 patients with more than 3 g/dl of monoclonal serum protein and more than 10% marrow plasma cells who have remained stable from 5 to 16 yr without treatment and have designated these patients as having SMM. We also have encountered 7 patients with Bence Jones proteinuria (>1 g/24 hr) who remained stable for more than 7 yr without treatment or development of MM or amyloidosis. None of these patients developed acute leukemia despite long periods of follow-up. However, treatment is ultimately required for a significant fraction of patients who fail to meet the criteria for MM initially but who develop overt MM during follow-up. In some, particularly those with SMM, the interval may be less than 1 yr. In others (for instance, many with MGUS), the interval to MM development may be 5–10 yr or more or as much as 24 yr.

Attempts to resolve the problem of differentiating MM from MGUS, including discriminant analysis of laboratory features, still leave some patients in whom the diagnosis is uncertain. Although plasma cells in patients with MM show morphological atypia, patients with MGUS and SMM also may show atypia, and most agree that an atypical plasma cell does not define MM. Morphological refinements, including measurement of nucleolar size and assessment of nuclear
cytoplasmic asynchrony,\(^1\) have been proposed to help differentiate MGUS from MM.

In our study, serum and urine monoclonal protein levels, percentage of plasma cells, and plasma cell nucleolar size, grade, and asynchrony were of limited use in differentiating MGUS from MM because of substantial overlap (Table 1 and Fig. 3). Moreover, these features provided practically no help in differentiating SMM from MM. Thus, clinical and morphological parameters do not help sufficiently to differentiate the patients with MGUS and those with SMM who have higher levels of serum and urine monoclonal protein and a higher percentage of plasma cells in the marrow from patients with MM. We have seen patients with as much as 5 g/dl of monoclonal protein in the serum and 20 g/24 hr of monoclonal protein in the urine, or with more than 50% plasma cells in the bone marrow, who have remained stable for years without treatment.

Others have found low plasma cell labeling indices in small numbers of patients with MGUS.\(^13\)\(^,\)\(^14\) In our study, the labeling index and percentage of plasma cells were the most reliable diagnostic parameters separating MGUS, SMM, and MM (\(p < 0.0001\)). The overall diagnostic accuracy using a plasma cell labeling index of greater than 0.4% to separate MM from MGUS and SMM was 83%. The plasma cell labeling index combined with the percentage of marrow plasma cells produced a linear discriminant function (Fig. 2) with a diagnostic accuracy of 92% or 95% if patients in whom MM developed in less than 6 mo were classified as having MM. More patients will need to be tested to determine the clinical usefulness of discriminant function analysis of multiple variables over the plasma cell labeling index alone. Most patients in the MGUS or SMM group initially had labeling indices of 0%. All patients considered initially to have MGUS or SMM with an index of more than 0.4% developed MM within 6 mo (see Fig. 3). These patients were clinically identified as having MM after an increase in serum monoclonal protein (two of three patients) and in percentage of marrow plasma cells (one of one patient tested), and after the development of lytic bone lesions (two of three patients) and of progressive osteoporosis with compression fractures (two of three patients). All other tests failed to discriminate these patients from patients with MGUS and SMM who did not develop MM (see Fig. 3).

Mean differences in labeling index between MGUS and SMM groups (0.1% and 0.1%, respectively) and the MM group (1%) represent a tenfold higher value in patients with MM and suggest a tenfold difference in percentage of cells in DNA-S phase. Assuming a plasma cell number of \(5 \times 10^5\) for MM and \(5 \times 10^3\) for MGUS, patients with MM would have a 2 log higher number of cells in DNA-S phase than would patients with MGUS. The difference in labeling index in untreated patients with MM compared to untreated patients with MGUS could account for proliferative differences in these groups and probably directly reflects plasma cell growth fraction differences between these two disorders.

The labeling index is a reliable method that can be used in MGUS and SMM when differentiation from MM by standard clinical criteria is difficult. Applied to patients with newly diagnosed monoclonal gammopathy, labeling index studies may spare some patients from treatment or may allow early diagnosis and treatment in others.

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