Usefulness and Reproducibility of Cytomorphologic Evaluations to Differentiate Myeloma From Monoclonal Gammopathies of Unknown Significance

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Abstract

We attempted to differentiate monoclonal gammopathies of unknown significance (MGUS) and multiple myeloma (MM) on morphologic grounds and to determine interobserver reproducibility of the differentiation. Cytologists blindly evaluated bone marrow smears from 154 patients with bone marrow plasmacytosis for the proportion of plasma cells with predefined cellular atypias. The single morphologic characteristic that most strongly differentiated MM from MGUS was the presence of nucleoli. The percentage of plasma cells, cytoplasmic contour irregularities, and anisocytosis also predicted a diagnosis of myeloma in multivariate analysis. Six cytologists independently evaluated 68 consecutive cases to determine sensitivity and specificity of these cytomorphologic features. The interobserver coefficient of variation for the plasma cell count was 33%. On consideration of the diagnosis, 36 of 41 MGUS cases and all 24 cases of myeloma were classified correctly. The use of a predesigned score system did not present such a bias, although it did not improve overall efficiency. The plasma cell count is the most predictive characteristic of myeloma from a cytologic viewpoint, but the interobserver variability is high. Interobserver variability is also high in the assessment of morphologic atypia, and atypical traits are not uncommon in plasma cells in MGUS.

The prognostic relevance of morphologic classification systems has long been recognized in the clinical approach to solid tumors, including lymphomas and multiple myeloma (MM).[1] In myeloma, it has been assumed that progression of the disease is associated with an increase in cytologic atypias of malignant plasma cells,[1-4] and many authors have attempted to correlate morphologic features with evolutionary disease and survival.[4-7] Some acceptable scores have been developed to differentiate monoclonal gammopathies of unknown significance (MGUS), smoldering myeloma, and evolutionary myeloma from a morphologic viewpoint,[5,8] but none has reached wide use in clinical practice. There are few reports of any of these scores being applied to a population other than that used to design the system,[9] and, to our knowledge, none of these studies has focused on the evaluation of interobserver variability. At present, diagnostic criteria of myeloma take into account only the proportion of plasma cells in bone marrow along with clinical, radiologic, and biochemical findings,[10,11] thus avoiding plasma cell morphologic criteria. We attempted to define to what extent MGUS and myeloma may be differentiated on morphologic grounds only and to what extent reproducibility from one observer to another is acceptable.

Materials and Methods

In the first stage (pilot study), cellular atypias of plasma cells from myeloma and MGUS samples were quantified by different cytologists. The characteristics most strongly associated with an MM diagnosis were identified. In the second stage (main study), samples from consecutive patients were

evaluated blindly by different cytologists according to previously defined criteria, and diagnostic accuracy and interobserver variability were evaluated.

Pilot Study

May-Grünwald-Giemsa–stained bone marrow smears from 154 patients with bone marrow plasmacytosis were selected to quantify the prevalence of each cellular atypia in bone marrow plasma cells and to define its relevance to differentiate MGUS from MM. Eighty-five cases (55.2%) corresponded to cases of MM and 54 (35.0%) to MGUS. Fifteen cases (9.7%), in which the plasmacytosis was reactive, were used as controls. Seven cytologists were asked to review the smears blindly and to evaluate the proportion of plasma cells presenting the following characteristics in a count of at least 100 plasma cells:

1. Nuclei: multinuclearity, central or eccentric position, chromatin maturity defined as nuclei with evident radial marks (cartwheel chromatin), nuclear membrane regularity, presence of nucleoli, and Dutcher bodies
2. Cytoplasm: presence of archiplasm (perinuclear hof), vacuolization, basophilia, flamed or irregular cellular shape, and cytoplasmic inclusions
3. Global characteristics: proportion of plasma cells in the smear, presence of clusters of plasma cells (a cluster was defined as a group of 4 or more adjacent plasma cells), clasmatosis (defined as atypical plasma cells with fragmented and spread cytoplasm), and the presence of anisocytosis or cells of abnormal size

Main Study

Eligible Patients

In the second stage, our aim was first to establish the sensitivity, specificity, and predictive value of bone marrow examination alone to predict a diagnosis of myeloma and second to quantify interobserver variability in the evaluation of the degree of infiltration and atypical characteristics of plasmacytosis in bone marrow smears. We studied 68 consecutive cases in which a bone marrow study was performed for one of the following reasons: (1) study of a monoclonal gammopathy, (2) study of osteolytic lesions, (3) pain with clinical suspicion of myeloma, and (4) normocytic-normochromic anemia with renal insufficiency or increased erythrocyte sedimentation rate. Patients with insufficient diagnostic data or less than 1 year of follow-up were excluded.

Sample Procedures

Bone marrow samples were aspirated by standard sternal or iliac puncture procedures and stained with May-Grünwald-Giemsa. If a patient fulfilled the inclusion criteria, 2 slides were given a numeric code. The name and clinical records of the patient were kept in the center of origin, and the 2 coded samples were sent with no other information to another center for evaluation. The cytologist of the center of origin was not to evaluate the center's own samples; therefore, each sample was evaluated by 6 cytologists from the other centers according to a predefined order of rotation under conditions that excluded knowledge of the patient's clinical information or evaluations by other cytologists.

Evaluation of Samples

Cytologists were to required to complete a form for all cases except those originating in their own centers. The following items were requested:

- A diagnosis of myeloma or MGUS according to their impression of the slides
- A percentage count of plasma cells in the sample
- Presence or absence of 3 predefined criteria of atypia **Image 1**: (1) more than 10% of plasma cells with an evident nucleolus of about 2 µm in diameter or more; (2) more than 10% of plasma cells with an irregular cytoplasmic contour or clasmatosis as previously defined; and (3) anisocytosis, defined as more than 20% of bone marrow plasma cells of a larger than normal size

Score-Based Diagnosis

The score-based diagnosis was established for each observer according to the proportion of plasma cells and the atypical traits observed regardless of the cytologist's previously held diagnostic impression. This score was based on the results of the pilot study and was applied to the samples of the main study only (see "Results"). The case was considered as myeloma if at least 1 of the 3 criteria of atypia was met and the number of plasma cells in bone marrow was more than 30%, if 2 criteria were met and the number of plasma cells in bone marrow was between 10% and 30%, or if all 3 criteria were met when the number of plasma cells was fewer than 10%.

Diagnostic Criteria

The Chronic Leukemia-Myeloma Task Force criteria[10,12] were considered the "gold "standard of the "true" clinical diagnosis **Table 1**. Patients with criteria of smoldering MM were not considered as a third group but were included as myeloma cases.

Final Review

In the third stage of the study, all the cytologically doubtful cases, those with excessive disagreement among observers in the proportion of plasma cells or atypias, or those in which the general criteria disagreed with the clinical diagnosis were evaluated together by the group and the clinical information was disclosed.

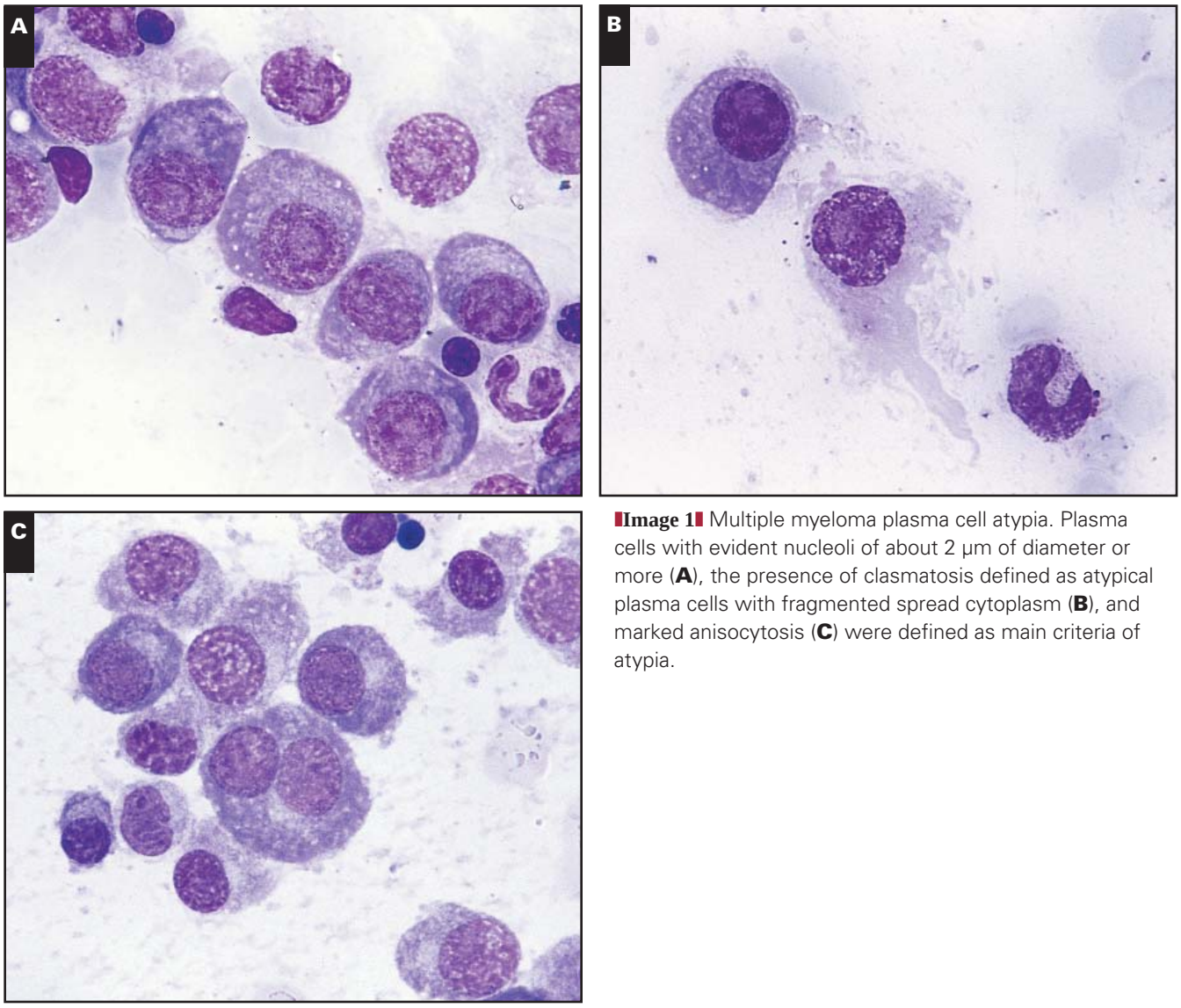

Image 1 Multiple myeloma plasma cell atypia. Plasma cells with evident nucleoli of about 2 µm of diameter or more (**A**), the presence of clasmatosis defined as atypical plasma cells with fragmented spread cytoplasm (**B**), and marked anisocytosis (**C**) were defined as main criteria of atypia.

Statistical Procedures

Information from the pilot study was evaluated by using the Student *t* test or the chi-square statistic to determine differences for each characteristic of atypia among MM and MGUS. Among statistically significant variables, logistic regression analysis was used to establish the independently significant characteristics associated with myeloma. Data from this study were used as a priori estimations of sensitivity and specificity for the main study and to establish an atypia score for the subsequent evaluation.

In the main study, the cases were entered randomly, and each case was evaluated by 6 investigators to obtain estimations of specificity, sensitivity, and interobserver reproducibility. Clinical data from the center of origin and the results of the cytologic evaluation from the other 6 centers were received and reviewed by an independent investigator. The plasma cell counts of each observer were included in a repeated-measures analysis of variance design. The clinical diagnosis was introduced as a covariate in the model. Differences among observers were tested for the series as a whole and separately for myeloma and MGUS cases. Interreplicate coefficient of variation for plasma cell counts was based on the sum of squares of the previous analysis of variance.[13] Reproducibility among observers in the presence or absence of atypias was contrasted using the Friedman test. The degree of concordance among observers was expressed as the mean of the Kendall coefficients of concordance for each pair. Finally, the McNemar test was used to evaluate bias in diagnosis or evaluation of atypias among observers. When all or 5 of 6 cytologists agreed on a diagnosis, the case was considered to have clear cytologic criteria of myeloma or MGUS. Otherwise (discordant diagnoses by 2 or 3 cytologists), the case was considered cytologically doubtful. The sensitivity

Table 1
Diagnostic Criteria Used to Establish a Clinical Diagnosis of Multiple Myeloma[10,12*]

Major Criteria

1. Plasmacytoma in a tissue biopsy specimen
2. Bone marrow plasmacytosis >30% in bone marrow aspirate or biopsy specimen
3. M protein concentration:
   Serum IgG >3,500 mg/dL (>35.0 g/L) or serum IgA >2,000 mg/dL (>20.0 g/L) or urinary Bence Jones protein (kappa or lambda) 1 g/24 h or more (in the absence of amyloidosis)

Minor Criteria

1. Osteolytic bone lesions
2. Bone marrow plasmacytosis between 10% and 30% in bone marrow aspirate or biopsy specimen
3. M protein at lower concentrations than in major criterion 3
4. Decline of normal levels of serum immunoglobulins:
   IgG <600 mg/dL (<6.0 g/L) or IgA <100 mg/dL (<1.0 g/L) or IgM <50 mg/dL (<0.5 g/L)

Smoldering Multiple Myeloma

Requires that diagnostic criteria for myeloma are met together with absence of symptomatology attributable to myeloma, <4 osteolytic lesions, absence of pathologic fractures, hemoglobin >10 g/dL (>100 g/L), normal calcemia, creatinine <2 mg/dL (<177 µmol/L), absence of infections attributable to hypogammaglobulinemia, and M protein IgG <7,000 mg/dL (<70 g/L) or IgA <5,000 mg/dL (<50 g/L)

* At least 1 major criterion and 1 minor criterion or 3 minor criteria must be met.

Table 2
Cytomorphologic Differences Between Plasma Cells in Bone Marrow Smears From Patients With Myeloma and Patients With Monoclonal Gammopathy of Unknown Significance (MGUS)[*]

	Mean Percentage of Plasma Cells (Range)		
Characteristic	**Myeloma (n = 85)**	**MGUS (n = 54)**	***P***
Central nucleus	16.4 (11-21)	12.7 (8-17)	NS
Multinuclearity	5.3 (3-7)	2.9 (1-4)	NS
No "cartwheel" chromatin	22.6 (17-29)	9.3 (7-33)	<.01
Irregular nuclear shape	3.2 (1-7)	0.7 (0-5)	.05
Nucleoli	32.9 (27-51)	11.5 (0-14)	<.0001
Dutcher bodies	0.5 (0-2)	0.6 (0-2)	NS
Cytoplasmic irregularities	44.7 (29-71)	22.3 (17-80)	<.0001
Vacuolization	30.2 (19-36)	49.2 (31-51)	<.001
Absence of archiplasm	36.4 (16-51)	28.1 (9-33)	NS
Cytoplasmic inclusions	5.2 (0-8)	1.4 (0-4)	NS
Overall anisocytosis	70.9 (65-73)	50.0 (30-75)	<.05
Plasma cells clusters	64.9 (58-81)	30.0 (13-41)	<.05
Overall plasma cells in bone marrow smear	48.6 (38-69)	10.2 (7-12)	<.0001

NS, not significant.
[*] Percentages in overall anisocytosis, plasma cell clusters, and overall plasma cells in bone marrow refer to smears globally. the remainder of the items refer to the proportion of individual cells in the smear. Comparisons by the Student *t* test.

and specificity of cytologic evaluation for myeloma diagnosis was based on this overall evaluation.[13]

Results

Pilot Study

As detailed in **Table 2**, myeloma cases presented a significantly higher proportion of plasma cells with nucleoli ($P < .0001$), with an irregular cytoplasmic contour or clasmatosis ($P < .0001$), without cartwheel appearance of chromatin ($P < .01$), plasma cells of larger size or anisocytosis ($P < .05$), and a higher frequency of plasma cells grouped in clusters ($P < .05$). Vacuolization was found more often in MGUS than in MM ($P < .001$). Reactive plasmacytosis control cases were undistinguishable from MGUS on morphologic grounds.

Considering only the cases with a proportion of plasma cells lower than 15% (19 cases of MM, 49 MGUS), the presence of nucleoli ($P < .036$) remained the only significant differential characteristic. When considered in a stepwise logistic regression analysis to predict the diagnosis of myeloma, the percentage of plasma cells ($P < .0001$), the presence of nucleoli ($P = .006$), cytoplasmic contour irregularities or clasmatosis ($P = .029$), and anisocytosis ($P = .025$) were the only independently significant variables.

Based on these results, 3 criteria of atypia were selected as candidates for a tentative scoring system, that is, the

presence of nucleoli, cytoplasmic contour irregularity and/or clasmatosis, and anisocytosis, and were used in the main study evaluation as described in the "Materials and Methods" section. Results from the pilot study allowed us to estimate a target of 60 random cases as necessary to obtain a range of no more than 10% in the evaluation of sensitivity and specificity with a priori estimates of more than 80% specificity, more than 90% sensitivity, and less than a 20% incidence of cases of myeloma in the random samples.

Evaluable Cases in the Main Study

Sixty-eight cases were reported from the 7 centers and included in the main study. Three were excluded from analysis because of a lack of definite clinical diagnosis, no follow-up after initial diagnosis, and inadequate samples for cytomorphologic evaluation. Of 65 valid cases, 24 (37%) had a diagnosis of myeloma. In 10 of these cases (42%), the plasma cell count in the bone marrow aspirate sample was less than 30%, and in 5 (21%), it was less than 20%. All 41 MGUS cases had bone marrow plasma cells less than 20%, although 7 (17%) had counts between 10% and 20%.

Plasma Cell Counts and Evaluation of Atypias

Plasma cell counts in bone marrow were significantly higher in myeloma cases ($P < .0001$) for each observer and for the mean among observers **Figure 1**. As detailed in **Table 3**, the interobserver coefficient of variation for the plasma cell count was 33% (46% for MGUS cases and 23% for myeloma cases). No significant differences were detected among observers in the plasma cell counts for the cases globally considered ($P = .092$), but significance was achieved for the subset of MGUS cases ($P = .03$).

As observed in the pilot study, the 3 selected criteria of atypia presented a significant concordance with a diagnosis of MM. Presence of nucleoli in more than 10% of plasma cells was the trait most strongly associated with MM ($r = 0.79$; $P < .000001$) and also the trait that presented the highest interobserver reproducibility (Table 3). A Friedman test for all cases revealed significant differences among observers in the evaluation of irregularities in cytoplasmic contour ($P = .021$).

Cytologic and Score-Based Diagnoses

On consideration of the diagnosis of the cytologists, 36 (88%) of 41 MGUS cases were classified correctly by at least 5 evaluators, although 2 were considered doubtful (disagreement by 2 cytologists). Five cases (12%) were classified incorrectly as myeloma. All 24 cases of myeloma (100%) were correctly classified, although 2 were doubtful (disagreement by 2 cytologists). The specificity, sensitivity, and efficiency of cytologic evaluations for the diagnosis of myeloma are summarized in **Table 4**. Although a statistically significant bias was detected between the individual evaluations and the clinical diagnosis for 2 observers, the global bias toward overdiagnosis of myeloma was not statistically significant.

The specificity, sensitivity, and efficacy of the cytologic diagnoses when based on the tentative scoring system are summarized in Table 4. Although the score-based system presented no bias among the observers or between the observers and the clinical diagnosis, its overall efficiency was not higher than the cytologists' diagnostic impressions.

Study of Discrepancies

In 9 cases (14%), a diagnostic discrepancy appeared between the clinical diagnosis and the observers' general criteria **Table 5**. Among 5 MGUS cases, 3 were considered to have an important degree of cellular atypia. This

Table 3
Differences Among Observers in the Proportion of Plasma Cells and Cytologic Atypia in the Samples of the Main Study

	Global (n = 65)	MGUS (n = 41)	Myeloma (n = 24)
Plasma cell count in smear			
Mean percentage (range)		7.5 (3-19)	41.5 (12-89)
Interobserver coefficient of variation (%)	33	46	23
Differences among observers (*P*)*	.092	.03	.314
Cases with >10% of plasma cells with nucleoli			
Kendall coefficient of concordance	0.72	0.77	0.62
Differences among observers (*P*)†	.38	.59	.12
Cases with >10% of plasma cells with clasmatosis or irregular cytoplasmic contour			
Kendall coefficient of concordance	0.46	0.31	0.68
Differences among observers (*P*)†	.02	.01	.51
Cases with anisocytosis			
Kendall coefficient of concordance	0.60	0.70	0.32
Differences among observers (*P*)†	.18	.37	.06

MGUS, monoclonal gammopathy of undetermined significance.
* From a mixed design analysis of variance.
† From a Friedman paired-variables test.

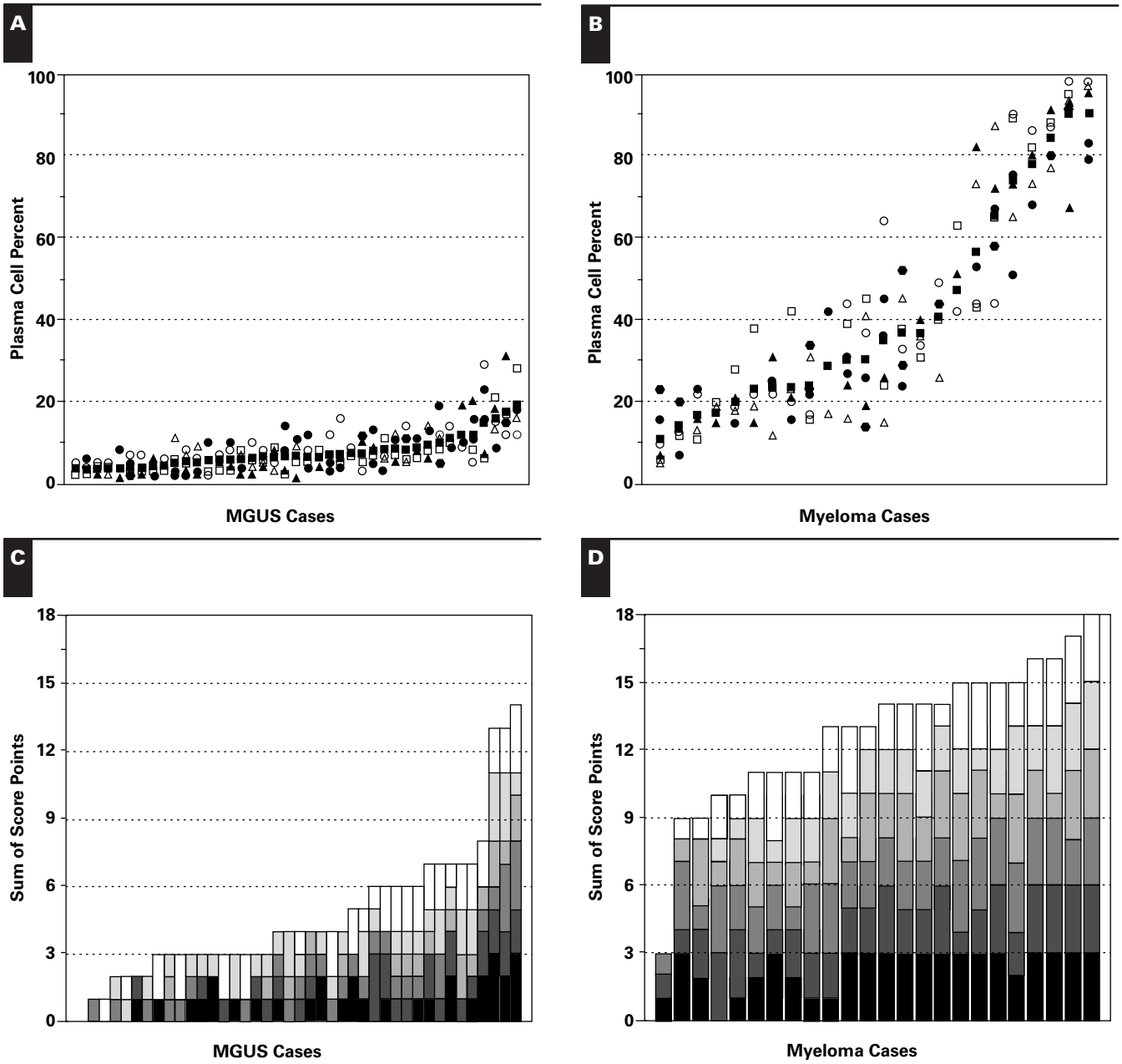

Figure 1 Individual evaluations of plasma cell counts and number of atypias for all cases. **A**, Bone marrow plasma cell counts for cases of monoclonal gammopathy of undetermined significance (MGUS). The mean is indicated by a closed square. **B**, Bone marrow plasma cell counts for myeloma cases. The mean is indicated by a closed square. **C**, Sum of score points for MGUS cases. **D**, Sum of score points for myeloma cases.

impression persisted despite disclosure of the clinical diagnosis (3 cases of MGUS of 1 or more years of evolution). A case of primary amyloidosis was misdiagnosed as myeloma despite atypias not being prominent, because of its high proportion of plasma cells. Finally, another case of MGUS showed remarkable heterogeneity in plasma cell concentration and morphologic features throughout the smear, leading to important discrepancies among individual observer results.

Four cases of myeloma (1 smoldering, 3 evolutionary), all of them correctly diagnosed by most cytologists, presented irregular distribution of plasma cells and different plasma cell morphologic features throughout the smear. This fact led to important individual differences between observers in plasma cell counts and evaluation of atypias. Remarkably, these cases did not satisfy sufficient criteria for a myeloma diagnosis when the diagnosis was score-based.

Table 4
Diagnostic Power of the Plasma Cell Count, Cytologists' Impression, and Application of Cytologic Score to the Main Study Sample

	Plasma Cells >30%	Cytologic Diagnosis	Score-Based Cytologic Diagnosis
Sensitivity	58.3 (44-72.6)	100 (85.7-100)	83.3 (69-97.6)
Specificity	100 (90.82-100)	87.8 (78.62-97)	90.2 (81-99.2)
Predictive value			
Of a positive result	100 (84.3-100)	82.8 (69.71-98.5)	87 (71.3-100)
Of a negative result	58.3 (46.3-70.3)	100 (88-100)	94.9 (82.9-100)
Overall efficiency	84.6 (77.3-91.9)	92.3 (85-100)	87.7 (80.4-95)
Cramer V	0.899	0.913	0.880
Uncertainty coefficient	0.810	0.830	0.763

Table 5
A Posteriori Discussion of Misdiagnosed or Undiagnosed Patients

Case No.	Mean Plasma Cell Count (Range) (%)	Mean* Score (Range)	Cytologic Diagnosis†	Score-Based Diagnosis†	Revision of Diagnosis
MGUS					
1.2	15.5 (9-21)	1 (0-2)	Myeloma 4/6	Undefined 3/3	MGUS nonevolutionary after 1-y follow-up
2.4	14.7 (6-29)	2 (1-3)	Myeloma 4/6	Myeloma 5/6	—
3.4	19 (12-28)	1 (0-2)	Myeloma 4/6	Myeloma 2/6	Primary amyloidosis
5.4	11 (9-14)	2 (1-3)	Myeloma 4/6	Myeloma 4/6	—
7.7	11.8 (9-19)	2 (1-3)	Myeloma 5/6	Myeloma 4/6	MGUS nonevolutionary after 3-y follow-up
Myeloma					
1.4	11.2 (5-23)	2 (0-3)	Myeloma 4/6	Myeloma 2/6	Smoldering myeloma
8.5	17 (13-23)	2 (1-3)	Myeloma 5/6	Undefined 3/3	—
8.18	23 (12-31)	2 (0-3)	Myeloma 4/6	Undefined 3/3	Evolutionary myeloma
9.3	23.5 (21-42)	1 (0-1)	Myeloma 4/6	No myeloma	Evolutionary myeloma

MGUS, monoclonal gammopathy of undetermined significance.
* Mean of scores rounded to integer value.
† Given as favorable evaluations/total.

Discussion

The plasma cell count in the bone marrow is a major criterion for the diagnosis of myeloma, but the diagnosis also relies on the presence of other criteria (ie, clinical symptomatology, osteolytic lesions, and immunoglobulin abnormalities). Morphologic atypia in plasma cells strongly correlates with the presence of evolutionary myeloma,[3,4,7] and when present in MGUS, it seems predictive of evolution to myeloma.[5] In clinical practice, most cytologists tend to suggest a diagnosis of myeloma when important plasma cell atypias are present, but the reliability of morphologic examination has been questioned by some authors.[14]

In our series of unselected consecutive cases evaluated for diagnosis of myeloma, as many as 42% of myeloma cases had a proportion of plasma cells in bone marrow aspirate samples less than 30%, and 21% had plasma cell counts less than 20%. These proportions of plasma cells in the smears from our random cases did not differ from other descriptions.[5,8] In consequence, although the plasma cell count remains the most important isolated characteristic to predict myeloma from bone marrow smears, its sensitivity to differentiate MGUS from MM is low, whether at a cutoff of 30% or 20%.

An important interobserver variability in plasma cell counts adds to this lack of sensitivity (coefficient of variation 33% in our study). This is especially important in MGUS cases, that is, in smears with low plasma cell counts. Thus, the distinction between MGUS and MM still requires the analysis of a combination of variables (bone lesions, serum or urine monoclonal immunoglobulin, symptomatology, and, most often, time).

It is a reasonable hypothesis that a qualitative or quantitative analysis of morphologic atypias may help to distinguish MGUS from MM. Our pilot study quantified the atypias most frequently present in myeloma plasma cells, which are, therefore, more relevant for diagnosis. However, these characteristics also were found in a variable proportion of nonmyelomatous plasma cells. Our morphologic observations coincide with other descriptions[8] and with observations based on other methods.

An interesting study by Kawano et al[15] identified plasmablasts as strongly positive for CD38 and negative for very late antigen 5 (VLA5) cells and found this subgroup of

plasma cells in higher proportions in advanced stage myeloma or plasma cell leukemia and in low proportions in smoldering myeloma or MGUS. Still, they found up to 20% of CD38++ VLA5– cells in some patients with MGUS or reactive plasmacytosis. They did not state to what extent CD38++ VLA5– plasma cells correlated with morphologic plasmablasts, but the study clearly suggests that atypical cells, both morphologically and immunophenotypically, are not rare in MGUS. Thus, assessment of the morphologic atypias of plasma cells to diagnose myeloma should be performed quantitatively, but our analysis demonstrates that this approach still presents important limitations.

Considering the mean of all cytologists' evaluations, 93% (38/41) of MGUS cases had no or 1 criterion of atypia, and myeloma cases had 2 or 3 criteria in 96% (23/24) of cases. Nevertheless, the differences among individual observers were considerable. Particularly, assessment of clasmatosis or cytoplasmic contour irregularities presented very weak reproducibility among observers for some subgroups of samples. Reproducibility in the assessment of anisocytosis was not high either. As a consequence, a relevant variability in plasma cell counts and variability in the evaluation of atypias among observers were detected. Our score was designed a priori from preliminary data and was the best-fit model in our pilot study, but when used in a different set of samples, the value of the scoring system dropped substantially. Subjectivity arises directly from evaluation of plasma cell counts and atypias; therefore, any cytomorphologic score is likely to present important drawbacks and is unlikely to improve the diagnostic power if evaluated in different sets of samples and by independent observers. Nevertheless, when the scoring system was used, less interobserver bias was detected, and the tendency to overdiagnose myeloma disappeared. Some published scores consider and differentiate as many as 4 or 5 degrees of maturity of plasma cells[1,3,7] to be applied, thereby imposing severe restrictions on a uniform application among different observers. Proposals to distinguish among plasmablasts and other neoplastic plasma cells[4,6] seem more realistic. Nevertheless, interobserver reproducibility to classify cells as plasmablasts should be assessed carefully when including their presence as a prognostic factor in multicentric studies.

There is some evidence that biopsy specimens improve the differential diagnostic power of cytology.[5,16] Although biopsy specimens might have proved useful in defining characteristics of the borderline MGUS cases in our study, all 24 cases of myeloma were classified correctly, and only 2 were considered doubtful (2 cytologists disagreed) on cytologic examination only. It is not clear that biopsy specimens would have overcome the problem of irregular infiltration by myeloma in different bone marrow areas. A precise role for bone marrow biopsy is still to be established in the diagnosis of MM. Immunophenotypic data (ie, kappa and lambda expression) reliably differentiate reactive plasmacytosis from a clonal condition but would not differentiate MGUS from MM. There is some recent evidence[17] that plasma cells from MGUS and MM might present some differential immunophenotypic characteristics. Were these data confirmed, the phenotype of plasma cells would emerge as a basic tool to differentiate MGUS from MM.

Clonal circulating plasma cells in different concentrations have been associated with MGUS, smoldering myeloma, and evolutionary myeloma,[18-20] offering the possibility of an objective and much less aggressive approach to the differential diagnosis. The clinical presentation of multiple myeloma has tended to shift from overt to indolent symptoms. Some patients whose symptoms do not fulfill classic myeloma diagnostic criteria at the time of their first evaluation might actually have indolent MM. Plasma cells with abnormal morphologic features, increased labeling index, aneuploidy, or other chromosomal abnormalities and histologic evidence of abnormal bone remodeling suggest a diagnosis of MM, but it will be important to standardize them if they become part of new diagnostic criteria. On the other hand, myeloma tended to be overdiagnosed in our study, implying that detection of early myeloma cases would not be the relevant problem from a cytologic viewpoint. The lack of specificity of cytologists' evaluations may be due to the presence of a significant proportion of atypical plasma cells in MGUS and to clinical considerations. It could be hypothesized that if patients with myeloma were treated based on diagnosis and not on clinical progression or symptomatology, cytologists would be more conservative in their diagnosis.

From the review of misdiagnosed or undiagnosed cases, 2 conclusions can be drawn. First, a small but relevant proportion of MGUS cases present with important cellular atypias, and this fact does not imply a fast evolution to myeloma. Some of these cases show remarkable heterogeneity in plasma cell concentration and morphologic features throughout the smear; thus, extensive and careful review of different slides cannot be overemphasized in the cytologic evaluation of atypical plasmacytosis. Second, despite its subjectivity, a qualitative cytologic evaluation adds sensitivity to the simple plasma cell count. Four MM cases (1 smoldering myeloma, 3 evolutionary) were diagnosed correctly by most cytologists despite an insufficient cellular count or a lack of sufficient criteria when the diagnosis was score based. As a consequence, cytomorphologic examination remains a subjective but highly accurate tool. Borderline MGUS cases accounted for all false-positive cases found on morphologic grounds in our study.

Our study points out and quantifies to what extent morphologic examination or morphologic score-based

systems are inherently limited by subjectivity and, consequently. fail to maintain their initial efficiency in different series. Standard cytology continues to be the most rapid and accurate tool to diagnose myeloma in clinical practice; still it cannot substantiate a diagnosis of MM as an isolated exploration. Cytologic features should be used with caution in investigational settings, like defining prognostic groups in multicentric studies.

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