Bone marrow biopsy in monoclonal gammopathies: correlations between pathological findings and clinical data

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Abstract

Between January 1987 and October 1989, 561 consecutive untreated patients with monoclonal gammopathy of undetermined clinical importance (MGUS) (n = 295) or with multiple myeloma (n = 266) were evaluated in a multicentre trial. Both bone marrow biopsy and aspiration (performed at different anatomical sites) were required at presentation. Bone marrow biopsy data indicated that changes in bone marrow composition from MGUS to early multiple myeloma and to advanced multiple myeloma followed a precise pattern, including an increased percentage of bone marrow plasma cells (BMPC%), a shift from plasmocytic to plasmoblastic cytology, an increase in bone marrow cellularity and fibrosis, a change in bone marrow infiltration (becoming diffuse rather than interstitial), a decrease in residual haemopoiesis and an increase in osteoclasts. In multiple myeloma the BMPC% of biopsy specimens and aspirate were closely related, although in 5% of cases the difference between the two values was greater than 20%. Some histological features were remarkably associated with each other. For example, BMPC% was higher in cases with plasmoblastic cytology, heavy fibrosis, or reduced residual haemopoiesis. Anaemia was the clinical characteristic most influenced by bone marrow histology. The BMPC% was the only histological variable which affected the greatest number of clinical and laboratory characteristics, including, besides haemoglobin concentration, erythrocyte sedimentation rate, radiographic skeletal bone disease, and serum concentrations of monoclonal component, calcium, β2-microglobulin and thymidine kinase activity. These data indicate that comparative bone marrow histology in monoclonal gammopathies has clinical importance.

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Multiple myeloma (MM) is a rather unique malignant disease in that the overall neoplastic burden can be calculated or, more simply, approximated from the serum or urine monoclonal component.[1] How skeleton, kidney, and haemopoietic marrow are affected is described by a series of simple radiological and laboratory data, including skeletal *x*-ray lesions, blood urea and nitrogen concentrations or creatinine concentration and Bence-Jones proteinuria, haemoglobin concentration, and percentage of plasma cells infiltrating the bone marrow (BMPC%) on bone marrow aspirate.[2] These variables have all been used for staging the disease at diagnosis,[3-5] which, in turn, defines prognosis. The ease with which multiple myeloma can be studied justifies further attempts to improve the definition of disease activity and associated changes.

A bone marrow biopsy specimen offers a better definition of bone marrow characteristics than bone marrow aspirate because it allows a greater number of variables to be obtained.[6] Besides BMPC%, the degree of plasma cell anaplasia,[7-9] and osteoclast activation[10 11] have been reported to be of clinical relevance, but only recently Bartl *et al* retrospectively included bone marrow biopsy variables into a multiple myeloma staging system.[12-14] The drawbacks of this system, however, are that the proposed criteria for defining these variables are rather complicated and central reviewing of the slides is required.

In a prospective multicentre trial for the study and treatment of monoclonal gammopathies a bone marrow biopsy specimen showing >20% BMPC% was one of three prerequisites for diagnosing multiple myeloma (the two others were the presence of serum or urine monoclonal component and of multiple osteolytic lesions unexplained by other causes: two criteria out of the three had to be satisfied). A simple and (we believe) accurate protocol for evaluating histological variables in bone marrow biopsy specimens was prepared and used by participating centres. In this paper we report the feasibility of this attempt as well as the differences in histological characteristics observed at diagnosis between monoclonal gammopathies of undetermined clinical importance (MGUS) and multiple myeloma, and among multiple myeloma stages,[3] and the association between histological and presenting features in multiple myeloma.[3]

Methods

Between January 1987 and October 1989, 742 consecutive patients with untreated monoclonal gammopathies entered a cooperative protocol for the study and treatment of multiple myeloma, referred to as "MM Protocol

Table 1 Bone marrow histological variables to be evaluated

1	Per cent of bone marrow plasma cell (BMPC)*
2	Cytology
	Predominantly plasma cells (= plasmocytic cytology)
	Predominantly plasmoblasts (= plasmoblastic cytology)
3	Cellularity
	Reduced = bone marrow: fat ratio of < 1:2
	Normal = bone marrow: fat ratio between 1:2 and 1:1
	Increased = bone marrow: fat ratio of > 1:1
4	Fibrosis (Gomori's stain)
	Absent = no evidence
	Low = fine reticulin network
	Intermediate = multifocal or diffuse non-confluent fibrosis
	Heavy = severe, diffuse fibrosis, with or without areas of collagenisation
5	Type of BMPC infiltration
	Interstitial (with preservation of bone marrow structure)
	Diffuse (with generalised or patchy changes in bone marrow structure)
6	Residual haemopoiesis†
	Hypoplastic
	Normal
7	Osteoclasts
	Absent
	Present

*BMPC% was determined as the differential of at least 2000 nucleated bone marrow cells at × 480 magnification.
†For evaluating residual haemopoiesis, the overall cellularity and the aliquots of normal haemopoietic precursors and plasma cells had to be taken into account.

1987". It was coordinated by the Institutes of Clinica Medica II and Anatomia Patologica at the University and IRCCS Policlinico San Matteo, Pavia.

Diagnosis of multiple myeloma required the presence of at least two of the following three features: (a) serum or urine monoclonal component; (b) BMPC% greater than 20, determined on trephine bone marrow biopsy specimen (a bone marrow aspirate also had to be taken from a different anatomical site); (c) osteolytic lesions on plain skeletal *x*-ray. Patients with a serum or urine monoclonal component but neither of the other two criteria were considered to have a monoclonal gammopathy of undetermined clinical importance. For diagnosis of non-secretory multiple myeloma—that is, patients fulfilling only criteria (b) and (c)—biopsy specimens of a lytic lesion showing plasma cell infiltration was required. Other causes of increased marrow plasmocytosis, such as rheumatoid arthritis, chronic infections, collagen disease, carcinoma, lymphoma or leukaemia, aplastic anaemia, and of monoclonal gammopathy, such as serum sickness, tuberculosis, neoplasms, were excluded before multiple myeloma or MGUS were diagnosed. Precise criteria were also established for the diagnosis of solitary osseous or extra-medullary plasmocytoma as well as primary plasma cell leukaemia in a few patients.

EVALUATION OF BONE MARROW HISTOLOGY

As a bone marrow biopsy specimen was required at diagnosis, a protocol for the complete evaluation of bone marrow histology was prepared. Prerequisites for this protocol were, of course, that it should take into account published procedures,[6-13 15 16] but also that it be simple enough to be used in a multicentre trial without requiring central reviewing of slides.

The protocol was first discussed at a slide seminar and agreed on by all the pathologists from the various institutions. Table 1 lists the seven established histological variables which had to be evaluated (in a semiquantitative way) to describe bone marrow. Figures a–d show some representative patterns. A bone marrow specimen had to measure at least 20 mm^2 to be evaluable. Dehydration and plastic embedding of core biopsy specimens was advised, and pathologists were asked to omit responses on individual histological variables in "doubtful" cases.

The reproducibility of bone marrow biopsy examination was checked (in December, 1989) in two ways: First, we directly determined the κ index.[17] Pathologists participating in the study were independently given the slides of three representative cases prepared at the co-ordinating institution. Agreement of individual responses with those given on the same cases by the proposing pathologists (AC, AC, and UM) was determined so as to eliminate the proportion of agreement among observers due to chance. In fact, the κ index represents the ratio between the actual agreement beyond chance and the potential agreement beyond chance. A perfect agreement is reached when the index is $>80\%$, and a satisfactory agreement when it is $>40\%$.

Second, we compared the bone marrow biopsy data obtained at the coordinating centre (and analysed by the pathologists who had proposed the protocol) (45% of patients) with those obtained at the affiliated centres (55% of patients). This was aimed at ascertaining major differences in the distribution of histological features due to uneven interpretation of bone marrow morphology.

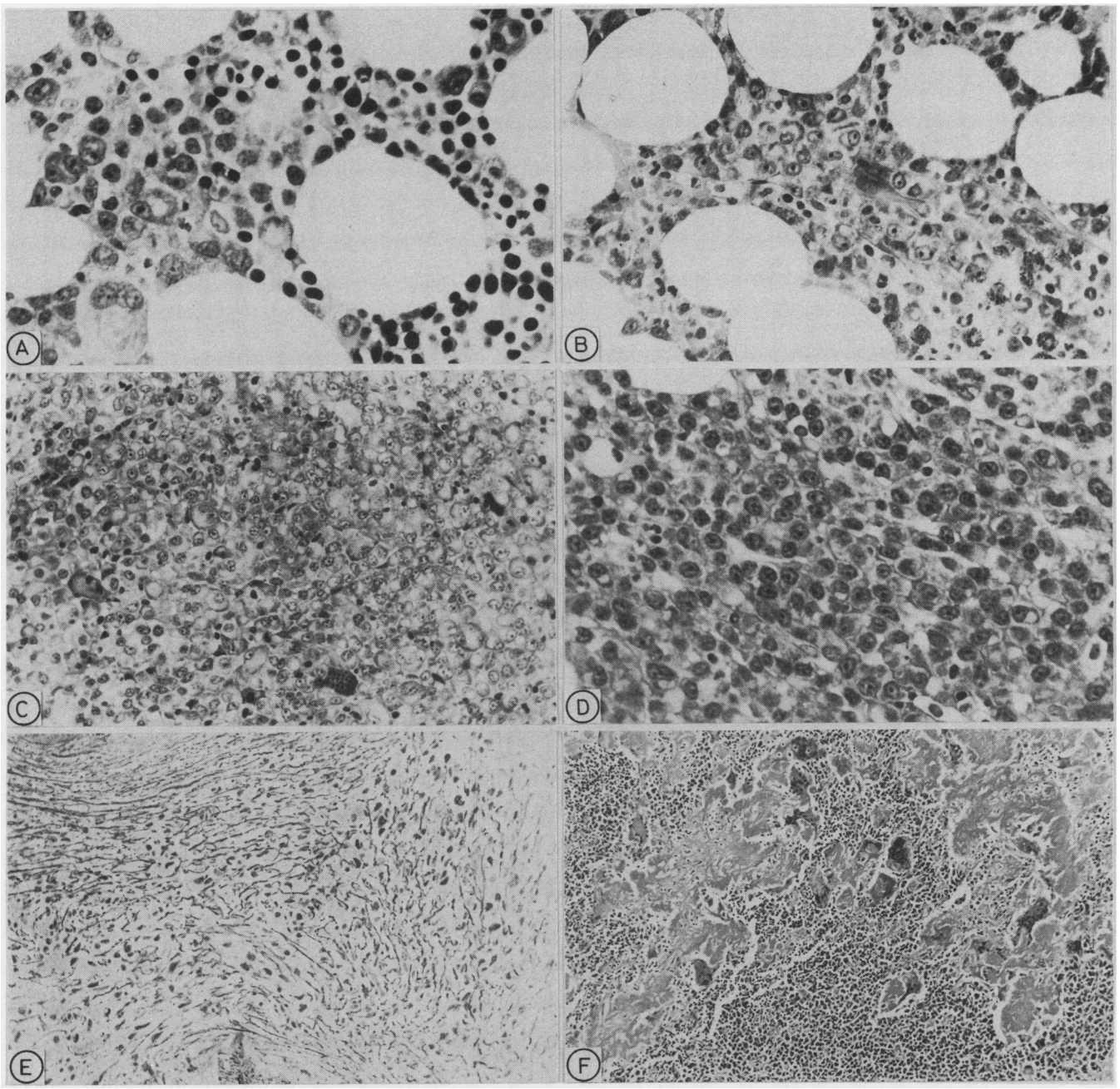
Basic data such as skeletal *x*-ray picture, haemoglobin concentration, serum or urine monoclonal component, calcium and creatinine concentration, as well as several additional laboratory variables, including erythrocyte sedimentation rate (ESR), white cell and platelet counts, serum concentrations of albumin, β2-microglobulin, and thymidine kinase[18] were collected at diagnosis for both patients with multiple myeloma or MGUS.

Differences between patient groups in the

Table 2 Clinical features at diagnosis of patients included in this study

	No of patients		
	MGUS	*Multiple myeloma*	*Total*
Patients studied	295	266	561
M/F	163/132	134/132	297/264
Age in years (median)	64	68	66
IgG	228	173	401
IgA	43	60	103
IgD		7	7
IgM	24		24
Light chain only		22	22
Non-secreting		4	4
Light chain type			
κ	170	164	334
λ	125	102	227
Stage I		56	
Stage II		77	
Stage III		133	

MGUS = monoclonal gammopathies of undetermined importance; multiple myeloma was staged according to Durie and Salmon[3].



Representative patterns of bone marrow disease in multiple myeloma, as classified in the protocol for evaluation of bone marrow biopsy specimens in this study. (a) Interstitial pattern of infiltration with atypical bone marrow plasma cells (BMPC) representing 20% of total nucleated bone marrow cells (plasmacytic myeloma); cellularity and residual haemopoiesis are within normal range (Giemsa). (b) Same as in (a) but with predominant undifferentiated BMPC (plasmoblastic myeloma). (c) Diffuse infiltration by well differentiated BMPC (90%), with few binucleated cells. Cellularity is increased but residual haemopoiesis hypoplastic (Giemsa). (d) Same as in (c) with undifferentiated (plasmoblastic) cytology. (e) Intermediate degree of fibrosis (Gomori). (f) Diffuse pattern of BMPC infiltration with numerous osteoclasts on residual bone trabeculae (Giemsa).

distribution of BMPC% (which is the only continuous variable we examined) were tested by the two-tailed Student's *t* test. For other histological variables (which are non-continuous) Fisher's exact test or Yates' corrected χ^2 test were used. Correlations among the different histological variables were investigated by the Pearson correlation procedure.

Differences in the distribution of clinical and laboratory data among groups of patients with different histological characteristics were tested using the two-tailed Student's *t* test. Testing for BMPC% was accomplished by dividing patients in two groups—those with higher and lower BMPC% than the median BMPC%.

Results

When this evaluation was carried out (December, 1989), the records of 561 of the 742 patients with monoclonal gammopathies recruited into the "MM Protocol 1987" between January 1987 and October 1989 were evaluable. Diagnosis was MGUS in 295 of 561 (52·6%) and multiple myeloma in 266 of 561 (47·4%). The general characteristics of these patients are listed in table 2. The remaining 181 are not included in this report because their records had not yet reached the coordinating centre (n = 146), or the data were unsatisfactory for diagnosis (n = 30), or the diagnosis was solitary osseous (n = 3) or extra-medullary plasmocytoma (n = 2).

Table 3 Distribution of bone marrow histological characteristics in multiple myeloma and MGUS and in multiple myeloma stages

No of patients with variable	*MGUS*	*Multiple myeloma*	*p Value*	*Multiple myeloma stage*			
				I	*II*	*III*	*p Value*
Median BMPC%	8·3	46	0·0001	23	41	58	0·0001
Cytology							
Plasmocytic	143	146	0·0001	38	45	63	0·005
Plasmoblastic	2	62		6	16	40	
Cellularity							
Reduced	18	27		5	11	11	
Normal	102	86	0·0005	24	25	37	0·2
Increased	21	56		10	14	32	
Fibrosis							
Low	136	127	0·0001	34	40	53	0·0009
Heavy	6	52		3	13	36	
Type of infiltrate							
Interstitial	90	98	0·0001	33	32	33	0·0001
Diffuse	22	90		7	22	61	
Residual haemopoiesis							
Hypoplastic	23	93	0·0001	10	29	54	0·02
Normal	127	93		32	25	36	
Osteoclasts							
Absent	102	91	0·0001	24	28	39	0·06
Present	13	56		10	14	32	

A bone marrow biopsy specimen was obtained at presentation in 382 of 561 (68·0%) patients, more often in multiple myeloma (217 of 266, 82·0%) than in MGUS (165 of 295, 55·9%). A bone marrow aspirate (at an anatomical site different from that of the bone marrow biopsy) was obtained in 499 of 561 (88·9%) patients—namely, in 239 of 266 (90·1%) with multiple myeloma and in 260 of 295 (88·3%) with MGUS. Physicians often preferred a bone marrow aspirate to biopsy specimen when the diagnosis of multiple myeloma was regarded as unlikely on clinical grounds, and therefore histological data are absent in several patients with MGUS. In most of the 49 patients with multiple myeloma who did not undergo bone marrow biopsy the diagnosis had already been ascertained by the simultaneous presence of serum or urine monoclonal component and multiple osteolytic lesions.

Seven variables had to be evaluated in bone marrow biopsy specimens (table 1). The BMPC% was determined in all cases. Plasma cell cytology, cellularity, fibrosis, type of BMPC infiltration, presence of osteoclasts and residual haemopoiesis were evaluated, in decreasing order (in 96–67% of patients with multiple myeloma and in 88–68% of patients with MGUS).

Overall results of the bone marrow histology are reported in table 3. Due to the diagnostic criteria, the BMPC% was much greater in multiple myeloma (median value = 46%, range = 5·8–98%) than in MGUS (median value = 8·3%, range = 2·5–19·5%). With respect to MGUS, multiple myeloma also had a greater incidence of plasmoblastic cytology, increased cellularity and fibrosis, diffuse type of plasma cell infiltration, hypoplastic residual haemopoiesis and osteoclasts.

In 218 patients (82·0%) with multiple myeloma the BMPC% was determined on the findings of both bone marrow biopsy and imprint. Median BMPC% values were similar in biopsy specimens (46·2%) and aspirates (44·5%), and paired values were also closely related ($p < 0·000003$). The overall dispersion around the correlation line, however, was quite wide ($r^2 = 0·49$), and in 11 (4·6%) cases the difference in BMPC% between the two bone marrow sampling procedures was greater than 20% (21–90%). In 24 (7·4%) patients only one of the two procedures gave a BMPC% diagnostic for multiple myeloma—that is, a BMPC% greater than 20%. In these cases the highest BMPC count was the accepted value for diagnosis.

The pattern of histological changes from stage I to stage III multiple myeloma was very similar to that seen from MGUS to multiple myeloma (table 3). With respect to early multiple myeloma, in advanced multiple myeloma the BMPC% was higher, cytology was more often plasmoblastic, cellularity was increased, plasma cell infiltration was more diffuse, fibrosis heavier, residual haemopoiesis scantier and osteoclasts were more common.

No significant difference in histological characteristics was detected between MGUS and stage I multiple myeloma.

The reproducibility of data collected at the different centres by the individual pathologists was good. The κ indexes were between 45 and 75% for the seven histological variables considered (table 1). The two groups of patients—that is, those from the coordinating centre and those from the affiliates—were similar for clinical stage and the other main clinical characteristics. No difference in the distribution of any histological feature could be attributed to uneven interpretation criteria.

In multiple myeloma, some histological variables were related to each other. BMPC% was greater in patients with plasmoblastic than in those with plasmocytic cytology (62 compared with 38%; $p < 0·0001$), with heavy rather than low fibrosis (59 compared with 40%; $p < 0·002$), with diffuse rather than interstitial infiltration (62 compared with 31%; $p < 0·0001$), and in those with poor rather than normal or increased residual haemopoiesis (57 compared with 33%; $p < 0·0001$). In individual cases bone marrow areas more heavily infiltrated by plasma cells also had more fibrosis, or osteoclasts, or both. Severe fibrosis

Table 4 Distribution of bone marrow histological characteristics according to some laboratory variables in multiple myeloma

Variable	ESR	Haemoglobin (g/dl)	Monoclonal component (g/dl)	Serum calcium (mg/dl)	β2 microglobulin (μg/ml)	Serum thymidine kinase (U/μl)
BMPC <46%	69	12·0	3·1	9·5	6·2	7·7
	0·0001	0·0001	0·0001	0·001	ns	0·05
>46%	100	9·8	4·5	10·2	7·8	14·7
Cytology						
Plasmocytic	81	11·4	3·6	9·6	6·5	7·0
	ns	0·002	ns	ns	ns	0·02
Plasmoblastic	99	10·2	3·7	9·7	7·6	16·3
Cellularity						
Reduced	91	10·8	3·7	9·5	5·4	6·6
Normal	76 ns	12·1 0·02	3·4 ns	9·5 0·04	7·1 0·001	9·5 0·003
Increased	86	10·5	3·7	9·9	6·3	14·2
Type of infiltrate						
Interstitial	78	11·7	3·3	9·5	6·4	6·9
	0·05	0·001	0·006	0·05	0·05	0·05
Diffuse	91	10·7	4·1	9·8	7·2	14·7
Fibrosis						
Absent/low	79	11·6	3·4	9·6	5·2	7·1
	0·01	0·0001	0·003	ns	0·02	0·05
Intermediate/heavy	99	9·7	4·3	9·9	10·0	14·8
Residual haemopoiesis						
Hypoplastic	94	10·3	4·0	9·7	6·6	14·6
	0·001	0·0001	0·006	ns	ns	0·05
Normal	71	12·1	3·2	9·5	6·8	7·7
Osteoclasts						
Absent	82	11·5	3·4	9·6	5·8	10·0
	ns	0·05	ns	ns	ns	ns
Present	88	10·8	3·6	9·7	5·3	12·8

was also more often found in patients with plasmoblastic cytology ($p < 0·02$). Residual haemopoiesis was especially poor in patients with increased cellularity and diffuse type of infiltration ($p < 0·0001$).

Overall correlations between histology and clinical characteristics are summarised in table 4. The haemoglobin concentration was the laboratory variable most profoundly influenced by bone marrow histology in multiple myeloma. Anaemia was, in fact, more severe in patients with a BMPC% of >46 or with plasmoblastic cytology, abnormally poor or abnormally increased bone marrow cellularity, heavy fibrosis, interstitial infiltrate, poor residual haemopoiesis and the presence of osteoclasts. On the contrary, white cell and platelet counts were not related to histology.

The degree of bone marrow infiltration by plasma cells was the single histological feature which influenced the greatest number of clinical and laboratory variables. The BMPC% changed according to the severity of *x*-ray bone disease. BMPC% was 34% in patients with no lesions or osteoporosis, 49% in those with less than three osteolytic lesions in different sites of the skeleton, and 55% in those with more extensive bone disease ($p < 0·002$). Patients with a BMPC% of >46 also had higher serum calcium than those with lower BMPC% (table 4). No other histological variable was related to *x*-ray bone disease and serum calcium. Additional laboratory variables which were higher in patients with a BMPC% of >46 than in those with lower bone marrow infiltration were ESR and serum concentrations of monoclonal component, β2-microglobulin, and thymidine kinase.

A few other correlations between histology and laboratory variables in multiple myeloma are shown in table 4. There were no significant differences among histological characteristics depending on sex, age, heavy or light chain subtype, presence or absence of Bence-Jones proteinuria, or serum concentrations of albumin, blood urea and nitrogen concentrations and creatinine and alkaline phosphatase activity.

Discussion

In a multicentre trial the feasibility of examining bone marrow histology was evaluated to ascertain the histological characteristics of MGUS and multiple myeloma and multiple myeloma stages, as well as their influence on the clinical and laboratory presenting features of patients with multiple myeloma. A bone marrow biopsy study protocol was used that was thought to fulfil reported requirements[6-13 15 16] and be simple enough to be used without requiring a central review of the slides.

The first step was to obtain, in addition to a bone marrow aspirate, a bone marrow biopsy specimen, which is not a standard procedure for diagnosing and staging monoclonal gammopathies. Bone marrow biopsy was avoided in almost half the patients who were later diagnosed with MGUS (where multiple myeloma was considered to be unlikely on the basis of presenting clinical features). On the other hand, biopsy was performed almost as often as aspiration in patients with multiple myeloma (in 82% and 90% of cases, respectively), where, as a rule, it was avoided when the diagnosis had already been ascertained by the presence of a serum monoclonal component and osteolyses. This indicates that bone marrow biopsy is acceptable as a diagnostic procedure when a serious disease is suspected.

Despite the fact that the BMPC% from biopsy specimens and aspirates (taken from different anatomical sites in 218 patients with multiple myeloma) were closely related, the pattern of plasma cell infiltration in multiple myeloma can be patchy. In fact, the dispersion of paired biopsy and aspirate BMPC% values around the correlation line was quite wide and, specifically, in about 5% of cases the difference between the two values was greater than 20%,

and in about 8% of cases only one value was greater than 20% (which was the level required for diagnosing multiple myeloma in this study). In patients without monoclonal component or osteolysis, or both, where the BMPC% value is a prerequisite for diagnosis, obtaining two bone marrow samples is therefore probably advisable.

The BMPC% was determined on all bone marrow biopsy specimens, and other histological variables—that is, plasma cell cytology, bone marrow cellularity and fibrosis, type of BMPC infiltration, presence of osteoclasts and residual haemopoiesis—were established, in decreasing order, in 92–68% of the cases (pathologists were specifically asked to omit responses in doubtful cases). Data were reproducible as the κ index[17] was quite high and the distribution of histological characteristics was similar in the two clinically comparable groups of patients (the one examined at the coordinating institute and the other at the participating centres). This indicates that the protocol is generally suitable for use in a multicentre trial without requiring central supervision. Further exercise will improve the already good collection of data.

Changes in bone marrow composition from MGUS to multiple myeloma and from early to advanced multiple myeloma followed an identical pattern which included increasing BMPC%, a shift from plasmocytic to plasmoblastic cytology, an increase in bone marrow cellularity and fibrosis, bone marrow infiltration becoming diffuse (rather than interstitial), a decrease in residual haemopoiesis and an increase in the osteoclast number.

This pattern of histological progression already suggests that there are interrelations among at least some of the histological variables—that is, some of them are probably interdependent variables in multiple myeloma, and this was confirmed by direct statistics. For example, the greater the BMPC%, the more pronounced the fibrosis (and several pathologists reported that the patchiness of fibrosis is also increased in the most heavily infiltrated areas, where osteoclasts are more frequently found).[6 10 16 19] Another example is that patients with plasmoblastic cytology also have a high BMPC% as well as heavy fibrosis. An overall interpretation of these data is difficult and perhaps unnecessary. The fact that both high BMPC%[5 14] and plasmoblastic cytology[7–9] have already been recognised as characteristics of patients with advanced or aggressive multiple myeloma leads to the speculation that these features are mainly responsible for the other changes in bone marrow histology—that is, for the increase in bone marrow cellularity, diffuse plasma cell infiltration, fibrosis, reduction of normal haemopoiesis and osteoclastic activation.

Apart from the overall condition of the patients (stage of disease), the haemoglobin concentration was the clinical variable most closely associated with histological progression, while the white cell and platelet counts were not influenced. This suggests that myeloma cells affect erythropoiesis, but not myelopoiesis or megakaryocytopoiesis, probably by humoral factors. That physical displacement of haemopoietic cells can produce anaemia without causing leucopenia, and thrombocytopenia is unlikely, while the production of active lymphokines is a recognised ability of multiple myeloma cells.[20–23]

On the other hand, the BMPC% was the only histological variable that affected (besides haemoglobin concentration) the greatest number of clinical and laboratory characteristics. In fact, x-ray bone disease worsened and serum calcium increased the greater the BMPC%, which is in keeping with the fact that plasma cells secrete a number of humoral factors which stimulate locally osteoclast activity (osteoclast activating factor).[20–24] The ESR and serum concentrations of monoclonal component, β2-microglobulin, and thymidine kinase (thought to be relevant prognostic factors)[18 25–27] were also strictly related to BMPC%. Osteoclasts (and fibrosis) were more frequently found in patients with a high BMPC% and in more heavily infiltrated, patchy areas.

In conclusion, this study indicates that it is feasible to obtain data on monoclonal gammopathies from bone marrow histology in a multicentre trial, that these data have clinical relevance and can be used for staging multiple myeloma. Up to now only BMPC% and plasma cell cytology[5 13] had been proposed as staging variables (without being widely used in practice), and the data presented here confirm that they are useful. What other histological variables may be relevant for survival cannot be confirmed at present, as some of them could be mutually related and the follow up of patients was quite short. More definite information will come from an adequate follow up and a multivariate analysis of pertinent clinical, laboratory, and histological data.

The following members participated in this study: Ematologia Ferrara (Professor G L Castoldi, Professor R Spanedda, Dr A Cuneo); Patologia Medica V, Milan (Professor C Rugarli, Dr E Bucci, Dr M Tresoldi); Semeiotica Medica, Policlinico Gemelli, Roma (Professor B Bizzi, Dr G Nicoletti); Ematologia, Parma (Professor V Rizzoli, Dr L Craviotto); Clinica Medica, Divisione Oncologia, Modena (Professor V Silingardi, Professor R Piccinini); Oncologia, Como (Dr C Epifani, Dr F Alberio); Ematologia Niguarda, Milan (Professor F De Cataldo, Dr L Barbarano); Medicina II, Cremona (Professor E Bianchini, Dr S Morandi); Medicina I, Piacenza (Professor L Buscarini, Dr M Di Stasi); Medicina II, Legnano (Professor E Cassi, Dr A De Paoli); Medicina I, Magenta (Professor C Novi, Dr E Rinaldi); Medicina I, Melegnano (Professor U Visca, Dr M Ventura Paradiso, Dr O Pica); Semeiotica Medica, l'Aquila (Professor D Quaglino, Dr A De Pasquale); Medicina B, Varese (Professor A Venco, Dr G Pinotti); Medicina C, Varese (Professor H Salmimi, Dr N Brumana); Medicina I, Gallarate (Professor L Ghiringhelli, Dr A Ceriani); Ematologia, Pisa (Professor B Grassi, Dr M Petrini); Medicina I, Alessandria (Dr G Montanaro, Dr A Pagetto); Oncologia, Mantova (Dr E Aitini); Medicina, Biella (Professor S Fontana, Dr M Badone); Medicina, Voghera (Professor F Nicrosini, Dr P Cardellini); Medicina I, Massa Carrara (Professor F Bechini, Dr E Maneschi); Medicina, Somma Lombardo (Professor M Mainardi, Dr A Daverio).

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