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Differentiation of plasma cell infiltrates in the bone marrow

A clinicopathological study on 80 patients including immunohistochemistry and morphometry

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Summary. In 80 patients immunohistochemical, morphometrical and clinical studies were performed on routinely referred trephine biopsies of the bone marrow showing an abnormal increase in plasma cells. From the approximately determined density of plasma cell infiltrates two main groups were distinguished, the first with an involvement exceeding 20% and the second with less than 10% of the total marrow area involved. The first group ($n=30$; 324 ± 130 plasma cells per square millimeter bone marrow) consisted of patients with frank malignant myeloma (MM) by clinical and histomorphological diagnosis. The second group ($n=50$; 132 ± 54 plasma cells per square millimeter bone marrow) with plasmacytic differentiation of infiltrates, had to be further divided into one component with evidence for initial or residual MM following chemotherapy ($n=27$), another with obviously monoclonal gammopathy of undetermined significance – benign monoclonal gammopathy (BMG, $n=6$), and a final set of cases with a reactive plasmacytosis mostly associated with an inflammatory condition ($n=17$). There was an excellent agreement between the intracellular immunoglobulin staining as defined by the immunoperoxidase technique and the serum or urinary M-component detected by immunoelectrophoresis. In MM significant correlations were found between osteoclastic activity (number of osteoclasts specifically stained by acid phosphatase) per trabecular bone area, presence of lytic bone defects and the density of plasma cell infiltrates in the marrow. This latter feature corresponded well with the titer of secreted serum M-components measured by quantitative immunoelectrophoresis. Using morphological data alone, BMG cases could not be discriminated with any certainty from initial or residual plasmacytic MM. They consequently need a prolonged clinical follow up to clarify the nature of the lesions.

Key words: Plasma cell infiltrates – Bone marrow biopsies – Malignant myeloma – Reactive plasmacytosis – Benign monoclonal gammopathy – Immunohistochemistry – Osteoclastic activity

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Introduction

The diagnosis of malignant myeloma (MM) from bone marrow biopsies should cause no difficulties, provided the infiltrates involve more than 20% of the total marrow area, show a nodular-focal pattern or a plasmablastic differentiation. However, frequently there are specimens which display a discrete infiltrate (less than 10%) of well differentiated plasma cells. In these cases the clinical question of an initial or residual MM following chemotherapy (staging procedure) may arise, as may difficulties in distinguishing between monoclonal gammopathy of undetermined significance (benign monoclonal gammopathy – BMG) and reactive plasmacytosis (RP). Since in routinely referred biopsy samples, cryostat sections are not practicable and bone marrow aspirates may not be available, immunological methods have to be performed on decalcified and paraffin embedded specimens. For this reason we undertook a clinicopathological study with the aims of evaluating the immunological reactions in routinely fixed, decalcified and paraffin embedded bone marrow biopsies with an abnormal increase in plasma cells; the differentiation of plasma cell infiltrates, particularly in those cases with the clinical question of an initial or residual MM or a RP; findings suggestive of a so-called BMG in comparison with the clinically established diagnosis of MM; and correlations between plasma cell infiltrates, osteoclastic activity and amount of secreted M-components.

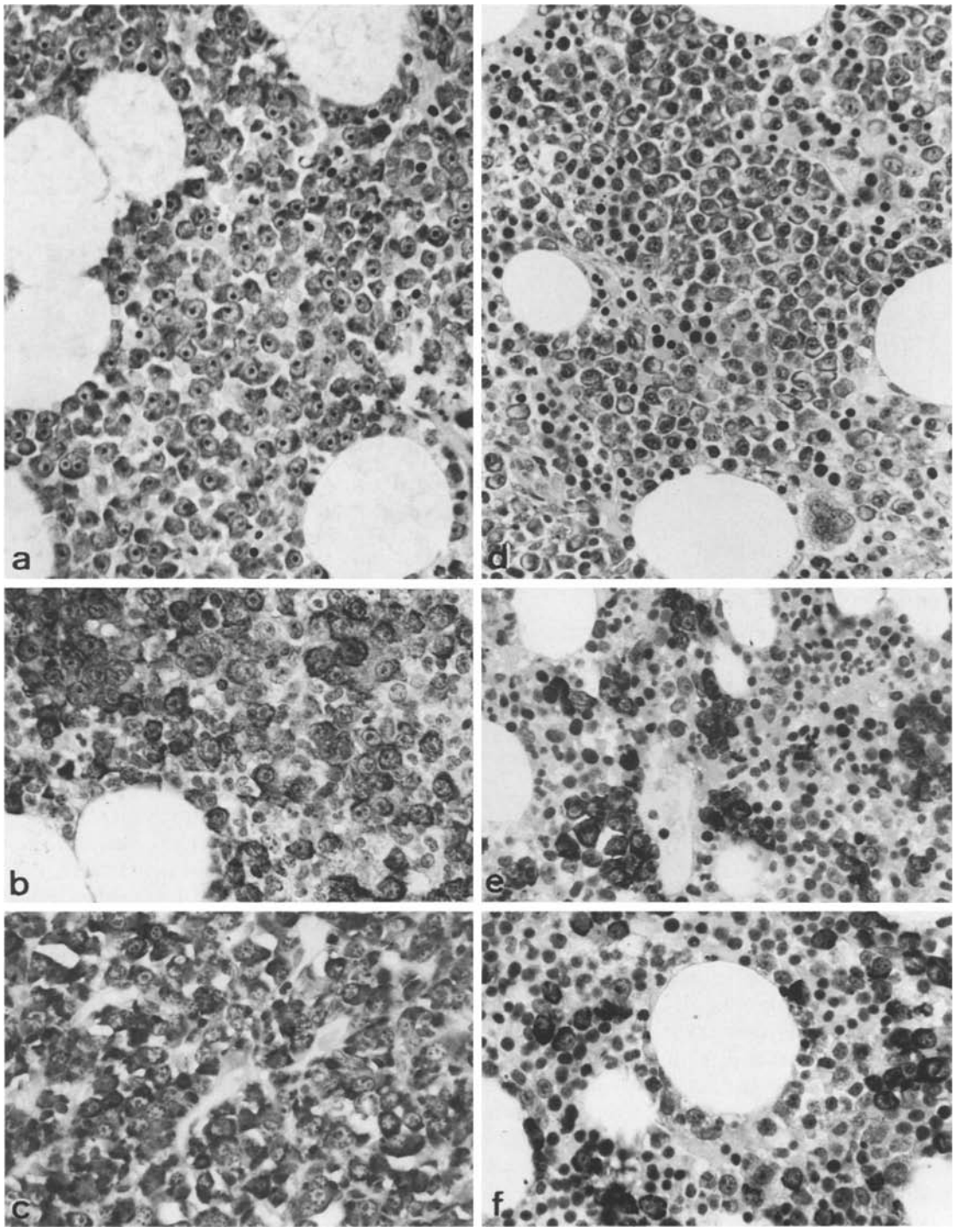

Fig. 1 a–f. Overt plasmacytoma (group I patients) with extensive involvement of the bone marrow showing a plasmablastic (**a**) or a plasmacytic-well differentiated (**d**) appearance. Immunohistochemistry reveals a positive reactivity against IgG (**b**) and lambda light chains (**c**) respectively IgA (**e**) and kappa light chains (**f**). **a–f** × 350; **a** and **d** Giemsa stain

Material and methods

All bone marrow biopsy specimens showing a conspicuous increase in plasma cells were recruited from our files. In the majority of cases these trephines had been referred to us with the clinical question of MM during the years 1980–1985. Very extensive and complete office and hospital records had been maintained on all patients, but particularly on immunological data and radiological findings.

Trephine biopsies were performed of the posterior iliac crest (Jamshidi and Swaim 1971). Fixation was in a solution containing a 1.5% formol-glutaraldehyde mixture and further

processing included decalcification (in EDTA for 3 days), paraffin embedding and employment of several staining methods (Giemsa, Gomori's silver impregnation, periodic-acid-Schiff – PAS –, methyl-green-pyronin, Prussian blue) and tartrate-resistant-acid phosphatase for the demonstration of osteoclasts (Schaefer 1984).

All sera and antisera for immunohistochemistry were diluted with phosphate-buffered-saline (PBS), pH 7.4. Rabbit heavy chain antisera (1:500), rabbit light chain antisera (1:300), normal swine serum (1:20), and rabbit peroxidase-antiperoxidase (PAP) complex (1:50) were obtained from Dakopatts (Copenhagen, Denmark). For immunohistochemical staining sections were dewaxed in xylene and brought to PBS, pH 7.4 through graded ethanols. Endogenous peroxidase was blocked by incubation for 30 min in methanol with 0.5% H_2O_2 and afterwards the sections were digested in 1% trypsin solution (Sigma Chemie, Deisenhofen, FRG) for 30 min at 37° C. For the demonstration of the various immunoglobulins the peroxidase-antiperoxidase (PAP) method originally described by Sternberger was used (Sternberger 1979). Initially sections were incubated with normal swine serum for 10 min, followed by the primary antibody (30 min), subsequently washed in several changes of PBS and again incubated with swine-antirabbit serum (30 min). After several washings with PBS sections were incubated with the rabbit peroxidase – antiperoxidase (PAP) complex (30 min), washed thoroughly in PBS and the peroxidase activity was then localized with the aid of 3-amino-9-ethyl carbazole (Graham et al. 1965). Following rinsing in distilled water sections were counterstained with haematoxylin and mounted in glycerol jelly.

Morphometric measurements were performed using a Zeiss-Kontron MOP-Videoplan with a program giving the mean values plus standard deviation for osteoclasts per square millimeter trabecular bone area (tartrate-resistant-acid phosphatase reaction without counterstain). Plasma cells were counted at 400 magnification with a 10 × 10 mm Zeiss grid ocular (diameter 0.46 mm) at least in 30 randomly selected fields and results expressed as number per square millimeter bone marrow area (Giemsa-stain).

Table 1. Survey of some morphological features of the bone marrow in 80 patients with an abnormal increase in plasma cells and consideration of the final clinical diagnosis. Mean density of plasma cells in all patients ($n=50$) of group II is 132 ± 54 per square millimeter bone marrow

	Group I	Group IIA	Group IIB	Group IIC
n	30	27	6	17
Plasma cells				
Approximate percentage	>20%		<10%	
Density per mm^2	324 ± 130	154 ± 73	188 ± 75	87 ± 34
Immunoreactivity	monoclonal	monoclonal	monoclonal	polyclonal
Pattern of infiltrates (ratio)				
focal-nodular	8/30	0/27	0/6	–
endostal	29/30	22/27	2/6	–
Differentiation (ratio)				
plasmacytic	17/30	27/27	6/6	17/17
plasmablastic	13/30	–	–	–
Increased cellularity of bone marrow (ratio)	23/30	9/27	3/6	8/17
Increase in reticulin fibers (ratio)	19/30	7/27	–	–
Final clinical diagnosis	Manifest (overt) plasmacytoma	Initial or residual plasmacytoma	Benign monoclonal gammopathy	Reactive plasmacytosis

Results

For practical reasons we divided our patients into two main groups according to the approximate extent of plasma cell infiltrates, established by gross calculation from 50 randomly selected cases. This pilot group revealed that the borderline between overt versus questionable myeloma infiltrates (not regarding cytological atypia or the topography of marrow involvement) ranged between a total density of 10 to 20% plasma cells per square millimeter of bone marrow. Consequently we distinguished group I patients ($n=30$) with more than 20% (324 ± 130 plasma cells/mm^2) and group II cases ($n=50$) with less than 10% (132 ± 54 plasma cells/mm^2) of total marrow involvement by plasma cells. The few cases with plasma cell counts lying in between these arbitrary limits were not further considered.

Patients in group I, showing conspicuous and often plasmablastic infiltrates were consistent with the morphological and clinical diagnosis of MM and served as controls (Fig. 1a–f) in comparison with the rather discrete alterations in group II cases. In consideration of clinical findings (see below) and immunological reactions group II could be differentiated into patients with an initial or residual MM following chemotherapy ($n=27$), a monoclonal gammopathy of undetermined significance – BMG ($n=6$) and finally a reactive plasmacytosis ($n=17$). Morphometric measurements of plasma cells together with a detailed evaluation of histopathology of these groups are given in Table 1. The various morphological features show a significant difference between overt MM (group I) and initial or residual MM (group IIA) as calculated by Student's t-test (level of significance $p<0.01$) with the exception of the variable endostal localization. It should be emphasized that in each single case the immunohistochemical reaction of the plasma cells (Fig. 1a–f) concurred with the clinical demonstration of certain light and heavy

Table 2. Results of quantitative and qualitative immunoelectrophoresis of serum and urine in 80 patients with abnormal plasma cell infiltrates in the bone marrow showing a monoclonal reactivity by histochemistry and a corresponding monoclonal gammopathy by clinical findings (normal serum levels of immunoglobulins: IgG 7.5–15.5; IgA 0.85–4.45 g/l). There was no case of a non-secretory malignant myeloma in our series

	Group I			Group IIA			Group IIB		
n	30			27			6		
	n	Serum titer (g/dl)	Urine light chains	*n*	Serum titer (g/dl)	Urine light chains	*n*	Serum titer (g/dl)	Urine light chains
IgG kappa	16	48±29	7	9	27±11	1	–	–	–
lambda	3	57±17	–	7	24±18	1	3	18±4	–
IgA kappa	3	18±15	1	4	15± 7	2	–	–	–
lambda	3	28±22	–	4	27±26	–	2	7±0.3	–
Unusual findings	1			1 biclonal			–	–	–
IgD lambda		9.0	1	IgG	23	1			
				IgA	12	–			
				lambda					
Light chains only kappa	1	–	1	–	–	–	–	–	–
lambda	1	–	3(2)	–	–	2	1	–	1
Final clinical diagnosis	Manifest (overt) plasmacytoma			Initial or residual plasmacytoma			Benign monoclonal gammopathy		

chains by serum and urine immunoelectrophoresis. For this reason these findings are not listed separately (for reference see Table 2). Moreover it has to be mentioned that immunoelectrophoresis was performed independently and with no prior knowledge of the results for the pathologists reviewing the slides. In comparison with our control group of manifest plasmacytic MM (group I – Fig. 1d–f) special regard was taken to establish any morphological lesion distinguishing group IIA patients (initial/residual MM – Fig. 2a–c) from BMG (group IIB – Fig. 3a–c). Both groups exhibited plasmacytic differentiation besides discrete marrow involvement, but in MM cases (group IIA) there was obviously a more pronounced endostal arrangement of plasma cells (Fig. 3d) and, particularly following chemotherapy, the development of reticulin fibers. In addition a pilot study of trephine biopsies was undertaken with morphometry from a randomly selected fraction of 10 non-treated patients with initial MM (group IIA) and the 6 cases with BMG (group IIB). In this study the number of plasma cells was evalulated for each case after staining with Giemsa, methyl-green-pyronin and compared with the positive immunoreaction (heavy and light chains). No significant difference between these counts was found using the various staining methods. Intracytoplasmatic precipitates of plasma cells seem to be slightly weaker in slides with a positive reaction towards heavy chains (IgG, IgA), especially if there was some background staining. In patients having received chemotherapy for MM (group I, $n=17$; Group IIA, $n=8$) a very few plasma cells (less than 2 per mm^2) with a polyclonal pattern of immunoreactivity were encountered infrequently. These minimal and scattered infiltrates may be regarded as reactive in nature and are probably associated with treatment. In context with these findings patients with a polyclonal reaction and thus a RP display a significantly lower density of plasma cells, a prominent perivascular arrangement or a randomly dispersed pattern, however, without an endostal (peritrabecular) localization (Table 1). These plasma cells often show a large size with an extensive cytoplasmic area and rarely 2 nuclei (Fig. 2d–f). Osteoclasts were detectable not in a randomly deployed arrangement along the endostal border of the trabecular bone, but apparently displayed an increase in number and prominent lining in the vicinity of plasmacytoma infiltrates (Fig. 3d, e).

Following morphological classification into 2 groups of patients with different involvement of the bone marrow by plasma cell infiltrates, clinical

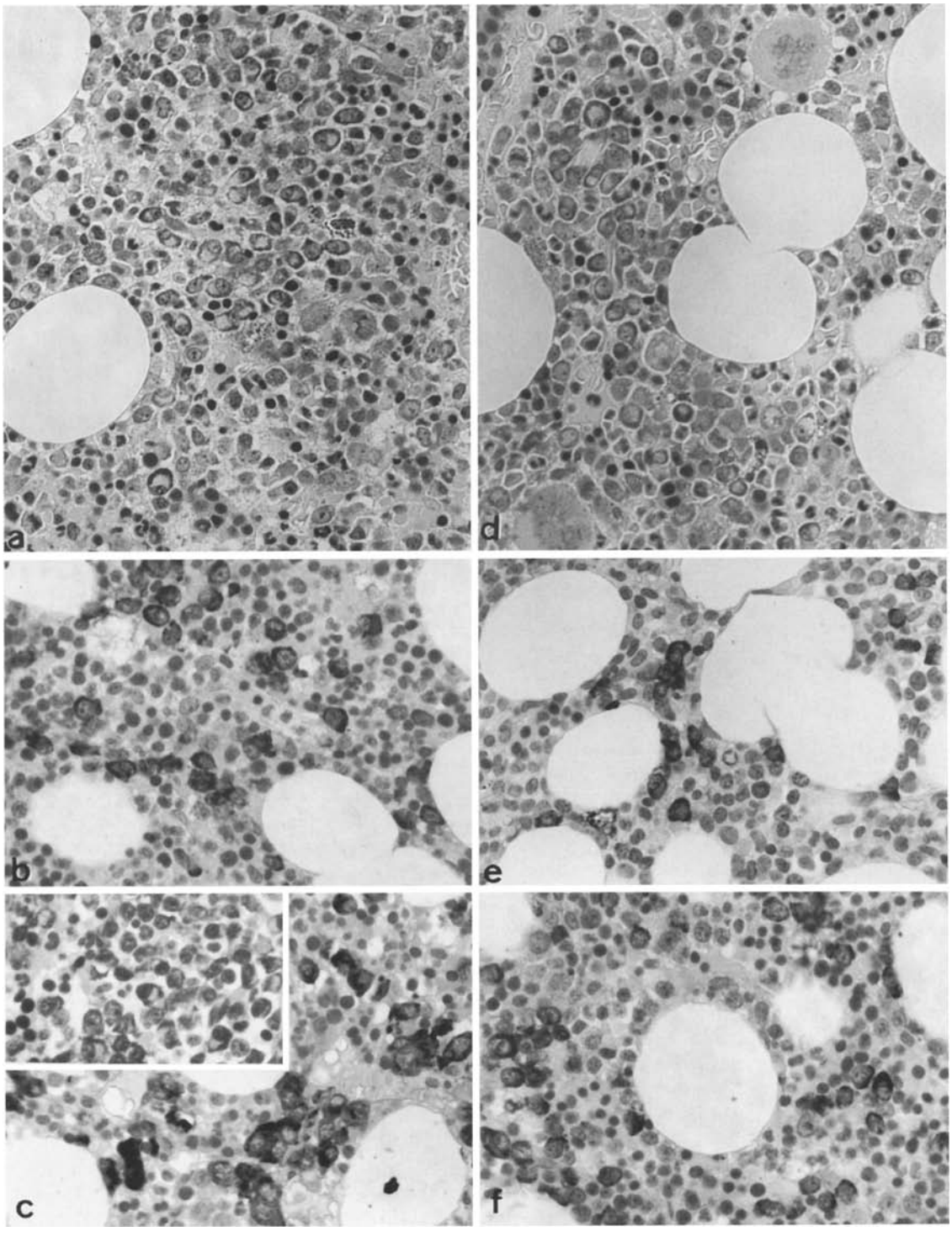

Fig. 2a–f. Initial plasmacytic malignant myeloma (group II A patients) versus reactive plasmacytosis (group II C patients). Small clusters of apparently well-differentiated plasma cells (**a**) showing a monoclonal pattern of immunohistochemistry with IgG (**b**) and kappa light chains (**c**) while lambda light chains are negative (*insect*). In contrast, in reactive plasmacytosis (**d**) mature plasma cells are deployed along a marrow vessel and reveal a positive staining against kappa (**e**) as well as lambda (**f**) light chains. **a–f** ×350; **a** and **d** Giemsa stain

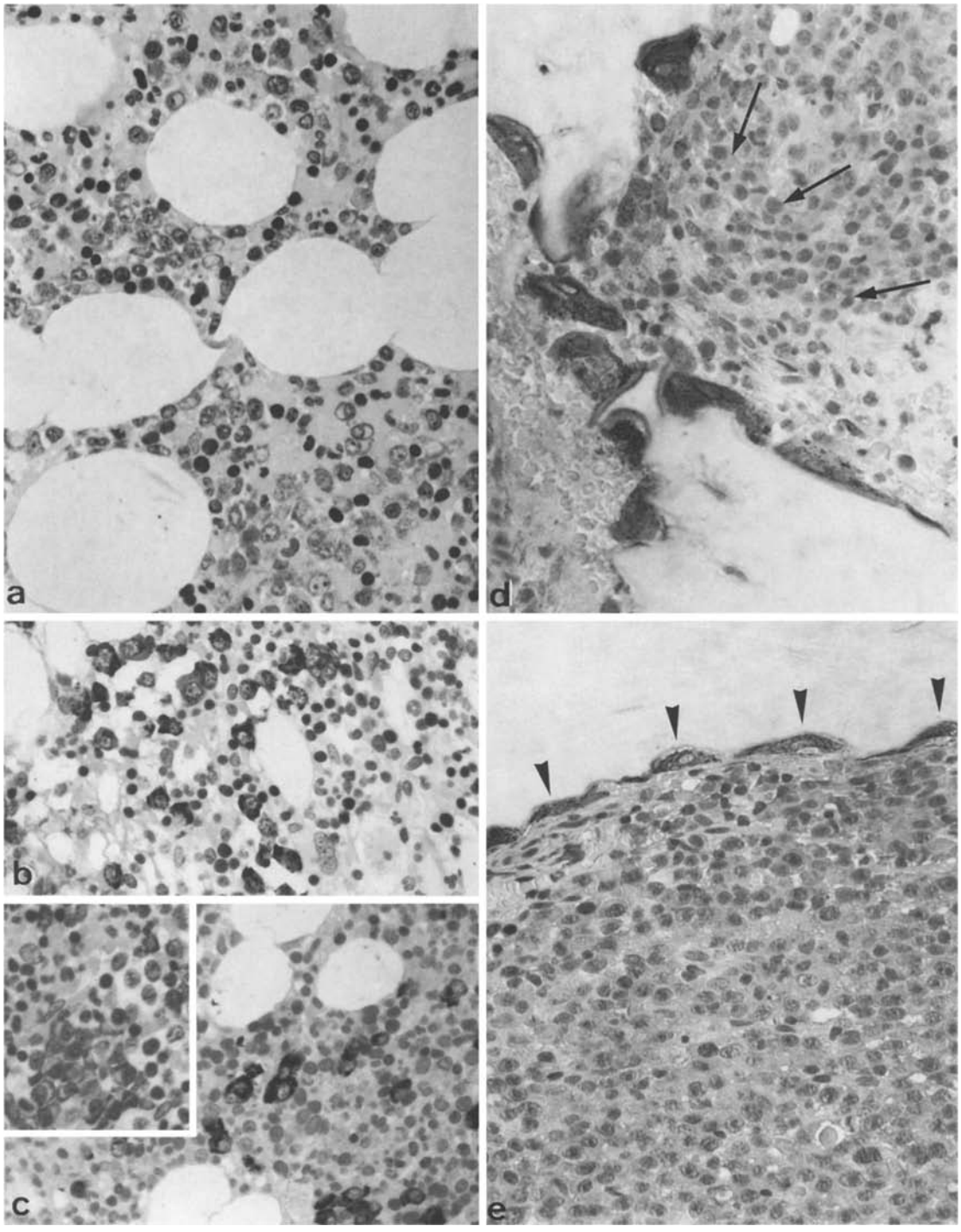

Fig. 3a–e. Benign monoclonal gammopathy (group II B patients) with scattered groupings of mature plasma cells (**a**) displaying a monoclonal pattern of immunohistochemistry against IgG (**b**) and kappa light chains (**c**) whereas lambda light chains are negative (*insect*). Osteoclasts present as either large multinucleated cells in a resorption lacunae (**d**) in the vicinity of focal endostal involvement by residual myeloma (*arrows*) or as inconspicuous lining of small elements along the trabecular bone (*arrow heads*) adjacent to gross plasmacytoma infiltrates (**e**) as shown by the acid-phosphatase reaction. **a–e** × 350; **a** Giemsa stain

Table 3. Reactive plasmacytosis (RP) – underlying conditions and serum immunoglobulin levels in 17 patients (group IIC)

Underlying disease by clinical diagnosis	*n*	Immunoglobulin levels (mean values) in serum (g/l) with standard deviation
1. Infection – septicemia	6	IgG: 18.3±13
2. Rheumatoid disease (arthritis)	5	IgA: 4.8± 5
3. Hodgkin's disease	2	
4. Mammary carcinoma	1	IgM: 1.3± 1
5. Crohn's disease	1	
6. not determined	2	

data and consequently the final diagnosis were found to be in concurrence with the histopathology (Tables 1 and 2). Serum and urine levels of immunoglobulins in patients with MM as well as BMG demonstrate the prevalence of IgG secreting plasma cells, 6 patients only with light chains MM (1 × kappa, 5 × lambda) and one case with IgD lambda and a biclonal IgA/IgG lambda MM, respectively (Table 2). There was no case of non-secretory MM in our series.

Follow-up studies of the patients with RP (group IIC) revealed various mostly inflammatory underlying conditions and a minimal or slight elevation of serum levels of immunoglobulins (Table 3).

In addition to the general agreement between the immunoreactivity of plasma cell infiltrates in the bone marrow and the qualitative assessment of serum and urine immunoglobulins there were other striking interrelationships. Significant differences (Student's *t*-test, $p<0.05$) were evident between overt MM (group I) and initial/residual MM (group IIA) when comparing counts for plasma cells, osteoclasts, serum levels of M-components (IgG, IgA) and the presence of lytic bone lesions (Table 4). No such differences, however, could be shown when considering the latter subgroup only (group IIA) and comparing it with the relevant values of the 6 BMG patients (group IIC). Moreover, a very high correlation ($r^2=0.95$, $p<0.001$) was found between the density of plasma cells (per mm^2 marrow area) and serum levels of immunoglobulins in each group of MM as well as with the osteoclast count (per mm^2 trabecular bone) and the occurrence of radiological bone defects (Table 4). To calculate an index of secretory activity of plasma cells, we assessed the ratios of serum levels of the M-components (in our patients IgG lambda-, kappa-component) divided by the mean counts of immunoglobulin containing plasma cells (per mm^2 bone marrow area). There was no significant difference between the plasma cells of overt MM (group I) and initial/residual MM (group IIA) except with the normal controls (Table 4). In BMG there were only 3 patients available for calculation of this index (Table 2) which revealed a considerably lower value of 0.098.

Table 4. Correlation of some morphological and clinical features in overt and initial/residual malignant myeloma (MM) including the so-called secretory index of plasma cells (see text) in comparison with controls

	Group I overt plasmacytoma	Group IIA initial/residual plasmacytoma	Controls normal bone marrow	Level of significance
n	30	27	20	–
Plasma cells per mm^2 bone marrow area	324±130	154±73	18±7	$p<0.0005$
Osteoclasts per mm^2 trabecular bone	7.17	2.88	2.59	$p<0.005$
Radiological lytic bone defects (ratio)	22/30	10/27	–	$p<0.001$
Serum level of M-component (g/l)				
IgG	49.3±27.2 ($n=19$)	23.5±12.5 ($n=16$)	7.5–15.5	$p<0.05$
IgA	22.4±17.6 ($n=6$)	18.7±16.3 ($n=8$)	0.85–4.45	$p<0.05$
Secretory index:	0.156	0.175	0.825	–

Discussion

The salient point of this study and related investigations is the distinction between discrete plasmacytic infiltrates (less than 10% of nucleated marrow cells) in the bone marrow which represent neoplastic ones (initial or residual MM – group IIA), from ones of indeterminable significance (BMG – group IIB) or from the frankly reactive type (RP – group IIC). This aim may be achieved only by employment of immunohistochemical methods to exclude RP. Discrimination between minimal MM-involvement, so-called smoldering MM and BMG depends on a thorough review of relevant clinical and morphological data and most impor-

tantly, follow-up studies. Morphological criteria used alone for the diagnosis of MM are not always reliable, since there may be a great variation in the appearance of individual marrow areas (Bartl et al. 1982). Moreover, focal or discrete infiltration of the malignant process may be present at the site taken for biopsy in early as well as in advanced MM. This supports the need to applying immunohistochemical methods to distinguish at least between the groups MM/BMG and RP, because quantitative aspects or cytological differentiations are sometimes misleading. Immunohistochemical methods have been successfully employed on paraffin embedded marrow aspirates (Taylor et al. 1978; Suzuki et al. 1984) and biopsies (Pinkus and Said 1977; Hitzman et al. 1981; Mullinik et al. 1985; Eckert et al. 1986; Hall et al. 1987; Lenormand and Crocker 1987), and are valuable especially in those cases where plasma cell infiltrates are discrete or composed of apparently well differentiated elements, and in those patients where a non-secretory type of MM was suspected. This suggests that in the majority of cases, only patients with difficulties in establishing an unequivocal diagnosis have been considered. In contrast, our relatively large number of 80 consecutively recruited cases with an increased amount of plasma cells in the bone marrow may be more representative of routinely referred specimens and their diagnostic problems. It is thus not surprising that we did not encounter a so-called non-secretory MM with a frequency of less than 1% in large series (Kyle 1975).

When applying immunohistochemical methods technical problems may arise in formalin fixed tissue samples, particularly in trephine biopsies following decalcification with EDTA. Occasionally an only weak reaction of plasma cells (mostly for heavy chains) is observed together with a disturbing background staining as was demonstrated in a comparative study (Mullink et al. 1985). In our experience this disadvantage can be successfully met by an enforced digestion of the sections with trypsin solution (Mepham et al. 1979; Jacobsen et al. 1980) and fixation by an aldehyde mixture, which in addition leads to excellent preservation of cytological details (Schaefer 1984). In this context it is noteworthy that in recent years the obvious tendency to embed bone marrow biopsies in plastic excludes the possibility of immunological methods and most histochemical reactions, unless special methods of fixation and resin embedding are employed (Takamiya et al. 1980; Beckstead et al. 1981; Blazek and Georgii 1984).

The differentiation between smouldering-initial MM and BMG remains controversial since there is no sharp line of distinction between these two entities (Ritzman et al. 1975; Lindström et al. 1978; Kyle et al. 1978). Histological as well as immunohistological methods fail to differentiate clearly early plasmacytic MM from this latter condition. The percentage of plasma cells is not a reliable criterion to distinguish between a benign and a malignant process (Kyle 1978; Turesson 1978; Bartl et al. 1982; Eckert et al. 1986). As can be inferred from the present study, only those few cases which exhibit a prominent endostal localisation of plasma cell infiltrates and a reticulin fiber sclerosis (Table 1) suggest the diagnosis of initial or residual MM. The results of immunological stainings in BMG are conflicting since a few authors apparently found a polyclonal pattern of reaction in some cases comparable with RP (Bartl et al. 1982; Eckert et al. 1986), but in contrast with the monoclonality of the serum or urinary M-component. Moreover, immunohistology yielded different ranges in kappa/lambda ratios for MM and BMG (Eckert et al. 1986). A distorted ratio of IgG/kappa to IgG/lambda secretory circulating cells, but a normal number of IgG/kappa plus IgG/lambda secreting plasma cells was further described in BMG by using a reverse haemolytic plaque assay (Shimizu et al. 1982). Therefore a shift in kappa/lambda ratio may indicate a transition from BMG to frank MM. Other authors (Greipp and Kyle 1983) reported significant cell kinetic differences between overt and smoldering MM and BMG. However, all these interesting results still need confirmation on a larger number of patients with a clear-cut diagnosis of BMG. At least the finding that the so-called secretory index of plasma cells may represent a distinguishing factor between MM and BMG (Suzuki et al. 1984) could not be reproduced in the present study which included many patients with conspicuous as well as discrete plasmacytoma infiltrates.

Evidence for the secretion of an osteoclast stimulating factor in MM has been found by isolation and culture of bone marrow cells (Horton et al. 1972; Luben et al. 1974; Mundy et al. 1974; Durie et al. 1981). In addition to the significant correlation between measured tumour cell number and tumour mass stage on overall survival (Durie and Salmon 1975) this factor may contribute to the invasiveness of MM by bone destruction (Durie et al. 1981). The presence of large multinucleated osteoclasts in increased numbers in resorption lacunae of the bony trabeculae adjacent to groupings of myeloma cells are well known features of the histopathology in this condition and have been

functionally associated with pronounced osteoclastic activity (Mundy et al. 1974; Bartl et al. 1982, 1984). However, precise measurements of osteoclasts are only feasible by employment of morphometry and histochemical methods, i.e. the tartrate-resistant acid phosphatase reaction (Schaefer et al. 1977). This procedure seems to be essential in order to reveal the many small uni-nucleated osteoclasts or their cytoplasmatic processes by the demonstration of specific enzymatic reactivity. Following this refined method we were able to show a positive correlation between the density of plasma cell infiltrates, number of osteoclasts and radiological presence of lytic bone defects in frank and initial/residual MM (Table 4). In this regard these findings confirm and extend the "in vitro" results on osteoclast stimulating factor in MM (Mundy et al. 1974; Durie et al. 1981). The lack of association between serum calcium concentration and these histomorphological features and lytic bone defects is noticeable. A complex homeostatic mechanism is in control of the serum calcium level. Consequently the calcium concentration will depend on the rate of entry into circulation, the capacity of the kidney to clear calcium and other factors which may be variable in individual myeloma patients.

Several morphological observations emerge from this study: – immunohistochemical methods may be successfully applied to routinely referred and processed bone marrow biopsies in order to distinguish between a monoclonal and polyclonal (reactive) pattern of plasma cell infiltrates; benign monoclonal gammopathy is not distinctive from initial or residual plasmacytic malignant myeloma from morphological aspects alone, but needs a prolonged follow up to ascertain its nature; in malignant myeloma there is a quantitative relationship between osteoclastic activity, presence of lytic bone defects and density of plasma cell infiltrates as well as the amount of secreted serum M-components.

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