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Histomorphometry of bone marrow biopsies in primary osteomyelofibrosis/-sclerosis (agnogenic myeloid metaplasia) – correlations between clinical and morphological features*

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Summary. Histomorphometry was performed on representative trephine biopsies of the bone marrow on admission of 50 patients (21 male, 29 female – age 67 years) with so-called primary osteomyelofibrosis/-sclerosis (OMF) not preceded by any other subtype of chronic myeloproliferative disorders. This study was firstly aimed at testing correlations between histological features (amount of haematopoiesis, cytological aspects of megakaryocytes, density of reticulin and collagen fibres and degree of osteosclerosis) and laboratory data, as well as spleen size and duration of relevant prediagnostic symptoms. Secondly, we concentrated on a discrimination of OMF patients into two subgroups according to bone marrow morphology and clinical variables. Statistical evaluation of histomorphometric variables and haematological findings disclosed that there was a progressive fibro-osteosclerotic process in the evolution of disease features. Increase in medullary fibrosis was significantly paralleled by an abnormal or pleomorphic megakaryopoiesis in the bone marrow: there was an increase in irregularity of perimeters for megakaryocytes and naked nuclei combined with smaller sizes of these elements including the nuclei. Additionally, there was a greater number of pycnotic bare nuclei. A number of morphometric features (density of fibres, degree of osteosclerosis, amount of haematopoiesis) were associated with corresponding clinical data (spleen size, length of preclinical history). By consideration of a set of basic histomorphometric variables our cohort of 50 patients could be divided into an early hyperplastic subtype with no or minimal medullary reticulin and another group with conspicuous fibrotic and osteosclerotic alterations of the bone marrow. It was noticeable that we found no significant correlation between amount of haematopoiesis or marrow cellularity with splenomegaly. This result suggests that splenic haematopoiesis (myeloid metaplasia) may represent an autonomous or neoplastic process and not only compensation for a failing fibro-osteosclerotic bone marrow.

Key words: Primary osteomyelofibrosis/-sclerosis – Agnogenic myeloid metaplasia – Amount of haematopoiesis – Content of fibres – Degree of osteosclerosis – Spleen size – Duration of preclinical symptoms – Histomorphometry – Clinical findings

Introduction

Among the various subtypes of chronic myeloproliferative diseases (CMPDs) agnogenic myeloid metaplasia (AMM) plays an ambiguous role comparable with "thrombocythaemia", since it may be divided into a so-called primary or "de-novo" form – primary (idiopathic) myelofibrosis – osteomyelosclerosis (OMF) and into a disorder complicating chronic myeloid leukaemia (CML) and polycythaemia vera rubra (P. vera) – postpolycythaemic myeloid metaplasia (Duhamel et al. 1970; Silverstein 1974, 1975; Devred and Diebold 1974; Laszlo 1975; Clough et al. 1979; Frisch and Bartl 1985; Ellis et al. 1986; Lazzarino et al. 1986; Thiele et al. 1988). When defining AMM as an entity not preceded by any other or allied subtype of CMPDs, thus implying primary OMF, certain problems exist regarding evolution of clinical and histological features in the course of this condition. Previous authors have referred to stages in bone

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marrow histopathology – early hyperplastic versus late fibro-osteosclerotic stages – based on sequential biopsies and have postulated a progression from the initial cellular to a burnt out or terminal stage with extremely developed osteosclerosis (Silverstein 1975; Lennert et al. 1975; Laszlo 1975; Frisch et al. 1984; Burkhardt et al. 1984, 1986; Georgii et al. 1984a, b; Lewis 1985). In this context the degree of splenomegaly has been shown to correlate with duration of disease and bone marrow cellularity as well as with fibrosis and osteosclerotic changes (Ward and Block 1971; Burkhardt et al. 1984; Geary 1985). Contrasting these findings, a number of authors were unable to demonstrate a significant relationship between duration of disease, medullary cellularity and increasing fibrosis of the bone marrow concurring with clinically estimated spleen size or weight and amount of extramedullary haematopoiesis (Bentley and Herman 1974; Wolf and Neiman 1985). In the view of these conflicting reports surrounding essential features of morphology and clinical data in OMF, the present study is aimed at delineating more precisely possible interactions between histopathology of the bone marrow and haematological parameters. For this reason we attempted to:

(1) Test and refine correlations between amount of haematopoiesis, cytological aspects of megakaryocytes, density of reticulin and collagen fibres and degree of osteosclerosis with clinical data, but particularly with spleen size and duration of relevant prediagnostic symptoms.

(2) Distinguish OMF into two subtypes according to bone marrow morphology and clinical parameters, that is, into an early hyperplastic versus an advanced fibro-osteosclerotic type.

A suitable approach to these problems has to include a morphometric evaluation of bone marrow features performed on representative biopsies of patients at presentation prior to any therapy, followed by a scrutinized assessment of corresponding haematological findings.

Table 1. Survey of haematological findings at presentation of 50 patients (21 male – 29 female, median age 67 years) with primary osteomyelofibrosis/-sclerosis (OMF)

Laboratory variables		Means ± SD	Ranges
Erythrocytes	$\times 10^{12}$/l	4.08 ± 0.9	2.32– 6.9
Haemoglobin	g/dl	11.27 ± 2.4	7.0 –17.4
Haematocrit	%	35.2 ± 6.9	20.3 –56.5
Leucocytes	$\times 10^{9}$/l	17.2 ± 14.1	2.7 –57.9
polymorphonuclear neutrophils	%	56.9 ± 19.1	11 –95
promyelocytes	%	2.25 ± 2.7	0–6
myeloblasts	%	1.7 ± 2.0	0–6
normoblasts	%	3.96 ± 6.0	0–25
basophils	%	2.11 ± 1.6	0–7
Thrombocytes	$\times 10^{9}$/l	449 ± 303	23–1360
Spleen size	(*)	7.0 ± 7.1	0–25
Liver size	(*)	2.1 ± 2.9	0–12
LAP score	(**)	97.4 ± 85	0–316
LDH	U/l	637.2 ± 169.4	116–1845
AP	U/l	246.4 ± 169.4	89–829

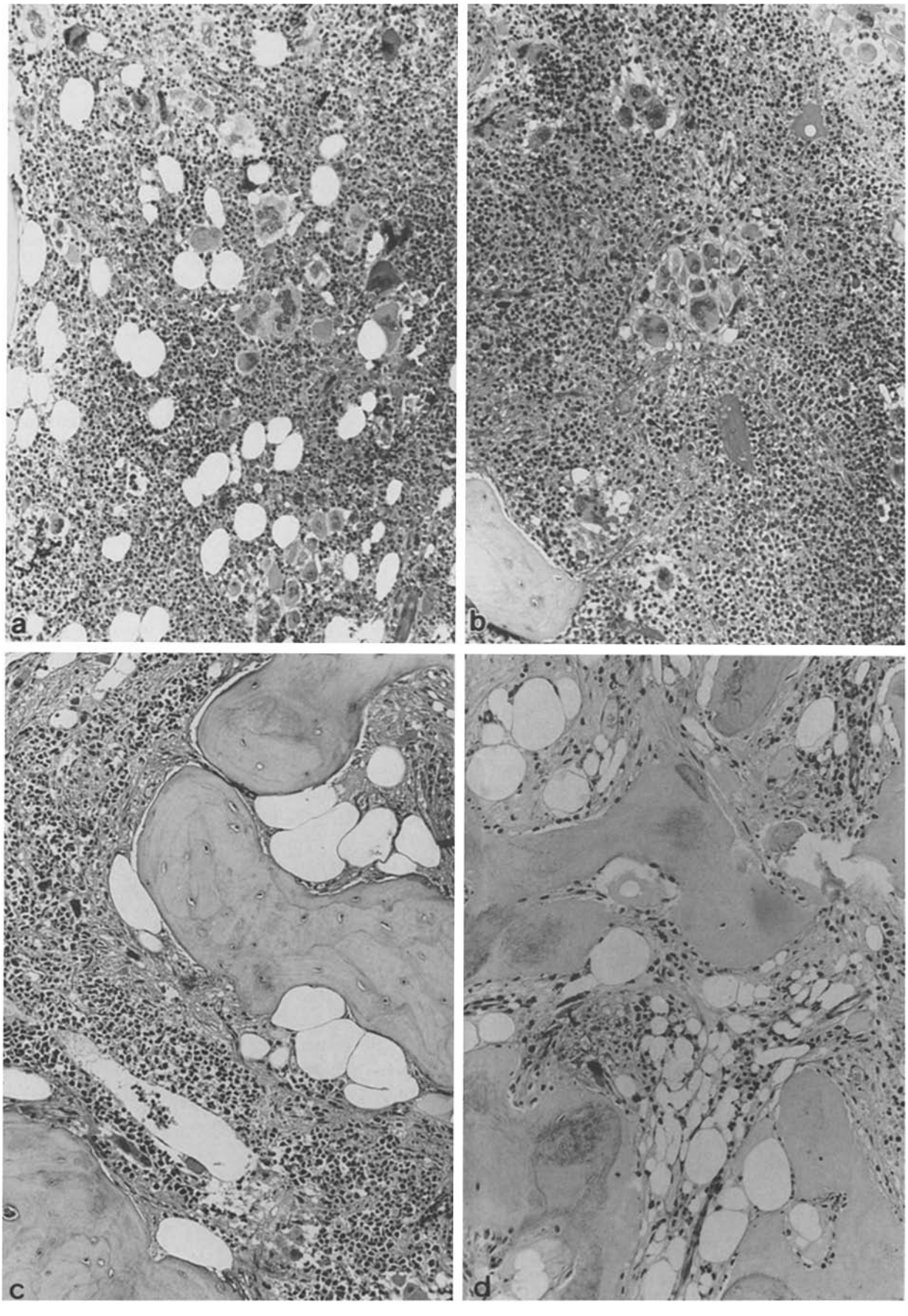
(*) cm below costal margin
(**) normal score 10–80

SD: standard deviation/LAP: leucocyte alkaline phosphatase
LDH: serum lactate dehydrogenase/AP: serum alkaline phosphatase

Materials and methods

A total of 50 patients (21 male – 29 female) with a median age of 67 years were enrolled in this study. All patients presented with the clinical signs and symptoms of OMF associated with characteristic haematological findings (Table 1). In agreement with historical data, cytological, cytogenetic and histological findings (including sequential biopsies in 6 patients), there was no evidence for any other subtype of CMPDs or a myelodysplastic syndrome. Duration of relevant symptoms (weight loss, fatigue, fever and night sweats, abdominal distress, easy bruising and thrombosis) prior to admission had lasted 22 ± 31 months (range 0–137 months). At the time of closure of this investigation (deadline April 15, 1987) 19 patients were dead and 31 alive. Causes of death (according to the data available from the death certificates) were lethal infections (pneumonia, septicaemia – $n = 11$) severe marrow failure including blast crisis ($n = 2$) and massive bleeding (cerebral and intestinal – $n = 3$). In 3 patients the causes of death were indefinite, particularly since no autopsy was performed. Bone marrow biopsies of 20

Fig. 1a–d. Survey of bone marrow features in OMF – haematopoiesis and bone remodelling. At onset a hypercellular bone marrow with a conspicuous myeloproliferation of pleomorphic megakaryocytes and a left-shifted neutrophilic granulo- and erythropoiesis may be present **a**. At a slightly later stage groupings of atypical megakaryocytes and interstitial oedema with a borderline increase in reticulin fibres is encountered **b**. Full-blown pictures of OMF are characterized by a patchy haematopoiesis, an obvious increase in reticulin, a few coarse bundles of collagen and a plaque-like apposition of primitive woven bone onto the spongious trabeculae **c**. Burnt out or terminal stages reveal a deformation and narrowing of the marrow spaces by pronounced osteosclerotic alterations of the trabecular bone, small residual islets of haematopoiesis and areas of adipose tissue **d**. **a–d** × 140; PAS reaction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patients (9 male – 11 female, median age 65 years) without any haematological disorder served as controls.

On admission of patients to our outpatient clinic trephine biopsies of the bone marrow were performed from the posterior iliac crest (Jamshidi and Swaim 1971). Fixation was done in an aldehyde solution for 12–48 h (2 ml 25% glutardialdehyde, 3 ml 37% formaldehyde, 1.58 g calcium acetate filled with distilled water to 100 ml) and further processing included decalcification for 3–4 days in 10% buffered EDTA, pH 7.4, paraffin embedding and employment of several staining methods (Schaefer 1984). These were applied for recognition of the various morphological features of the bone marrow: Giemsa – survey; periodic acid Schiff reagent (PAS)–megakaryocytes; naphthol-AS-D-chloroacetate esterase – neutrophilic granulocytes (negative for erythropoiesis); Gomori's silver impregnation – reticulin and collagen (argyrophilic) fibres. Additionally, for the detection of haemosiderin, the Prussian blue reaction was used. The tartrate-resistant acid phosphatase method was used for the identification of osteoclasts (Schaefer et al. 1977).

Morphometric evaluation was performed by a manual optic planimeter (MOP-A-MO1-Kontron) with a standard program set (Kontron software) on trephine biopsies with an area of 22.9 ± 12.8 mm^2. Counts for neutrophilic granulo- and erythropoiesis and megakaryocytes per square millimetre were measured at 500× magnification by calculation of the evaluable marrow area of the trephine biopsy (excluding areas of haemorrhage and artifacts as well as cortical and trabecular bone) and the total number of the corresponding cells (nucleated erythrocyte precursors, neutrophilic granulopoiesis and megakaryocytic elements). The megakaryocytes were classified into nucleated forms, anuclear fragments or extensions of cytoplasm and naked (bare – pycnotic) nuclei. Emperipolesis (internalization of haematopoietic cells) and mitotic figures were also evaluated (see also Table 3). A total number of 3543 megakaryocytes were counted in OMF and 1393 of this cell line in the control group of patients. After dividing the total area of the trephine biopsy into 5 segments of approximately the same size, 20 randomly selected megakaryocytes were measured in each field at a magnification of 1250× with determination of area, diameter, circumference and circular deviation (form perimeter). Additionally, the following parameters were calculated:

(1) Circular deviation (CD) of the perimeter for megakaryocytes and their nuclei was defined as $CD = 4\pi A/C^2$ (C = circumference and A = area), giving the value-1-respectively 100×10^{-2} for a circular shape and a lower factor indicating an ellipsoid outline or increased irregularity of the perimeter.

(2) Reticulin and collagen (argyrophilic) fibre content was measured in sections following silver impregnation (Gomori's stain) by counting the number of intersections (i) with the lines of a grid ocular at a magnification of 500× in 20 randomly selected fields free from cortical or trabecular bone (equalling 1.14 mm^2). The area covered by fat cells was substrated and the reticulin fibre density expressed as the number of intersections per square millimetre of fat-cell-free haematopoietic tissue (i/mm^2).

(3) An index for osteosclerosis (IOS) was determined by the ratio: areas of bone marrow to trabecular bone (mm^2) and frequently expressed as reciprocal value (1/IOS). Therefore a value of IOS = 1.0 indicates maximum osteosclerosis, whereas a value of IOS = 0.25 or less represents no apparent osteosclerosis.

The clinical, morphometric and haematological data were entered into the computer facility of the Institute of Medical and Statistical Documentation of the University of Cologne. The statistical analysis was executed by using the BMDP Statistical Software, Inc. (1964 Westwood Blvd., Suite 202, Los Angeles, California 90025, USA) for the Cyber series. To find the relationships between the histomorphometric, haematological and clinical variables we computed the correlation and regression coefficients. As an indicator for high correlation the p-value of $p < 0.05$ or less was demanded (Fisher 1972).

Results

There is a remarkable variety in the appearance of bone marrow features in patients with an established diagnosis of OMF. An overview of so-called early hyperplastic stages shows a conspicuous proliferation and clustering of megakaryocytes (Figs. 1a, b; 2a) which exhibit many abnormalities of maturation (Fig. 4c). Additionally neutrophilic granulo- and erythropoiesis seem to be left-shifted with predominance of numerous precursors. At this stage of OMF, which is assumed to present the initial lesion at onset of disease, there may be no increase (Fig. 2a) or an only minimal to slight deposition of reticulin fibres around the sinus walls and vascular structures (Fig. 2b). In contrast, in the clinically full-blown picture of OMF there is more patchy haematopoiesis (Fig. 1c), with groupings of atypical megakaryocytes which reveal a tendency to abnormal dislocation towards the peritrabecular (endosteal) generation zones of granulopoiesis (Fig. 4b, c). There is a striking increase in the density of argyrophilic fibres (Figs. 1c; 2c) with interspersed bundles of coarse collagen (Fig. 1c). The spongy bone displays plaque-like appositions along its endosteal border (Fig. 1c). More advanced stages show a reduction of haematopoietic marrow with replacement by adipose tissue. Furthermore, there is a bizarre endophytic growth of woven bone – osteosclerosis (Fig. 1d) and a dense meshwork of reticulin and collagen fibres, apparently in close association with the

Fig. 2a–d. Survey of bone marrow features in OMF – fibro-osteosclerotic changes (compare with Fig. 1 **a–d**. Initial stages show no increase in the medullary reticulin content, but a hypercellular bone marrow **a**. In slightly more advanced stages in the course of disease a minimal deposition of reticulin fibres along the sinus walls is noted **b**. Full-blown stages of OMF are characterized by a dense meshwork of reticulin, interspersed with coarse bundles of collagen fibres **c**. Terminal stages display overall collagen fibrosis replacing the neoplastic haematopoiesis and exhibiting a close association with the newly formed intramembranous bone or neo-spongiosa **d**. **a, b, d** ×140, **c** ×200 and insets ×350; **a–d** silver impregnation after Gomori

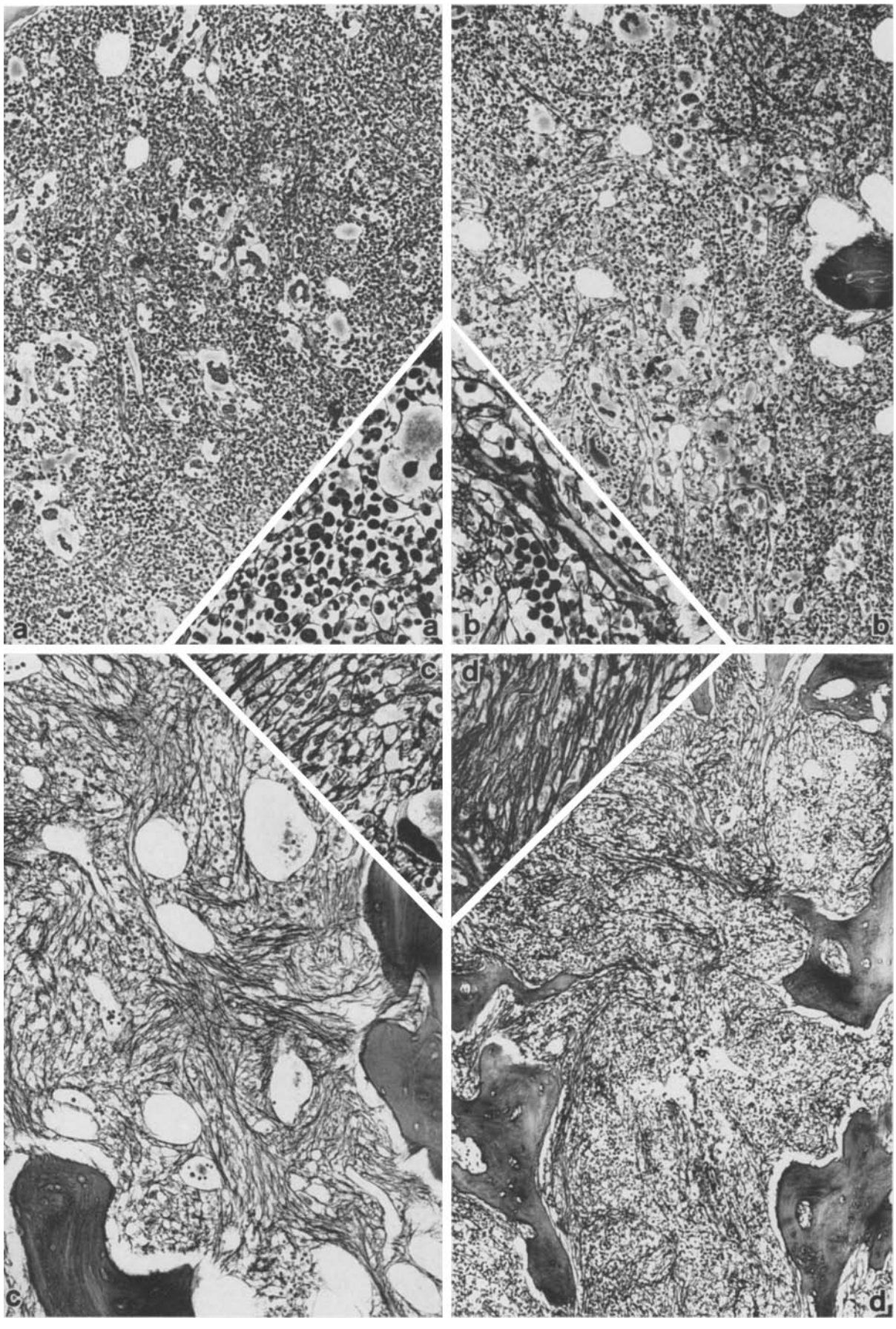
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Table 2. Histomorphometric features evaluated from trephine biopsies of the bone marrow at presentation of 50 patients with OMF (see also Table 1) in comparison with control cases

Parameter	Controls		OMF		Significance (p-value)
	means ± SD	ranges	means ± SD	ranges	
Area of biopsy mm^2	22.9 ±12.8	7.4 –64.8	20.9±12.7	7.5 –66.9	n.s.
Trabecular bone %	26.0 ±9.9	3.1 –42.5	31.2±11.1	7.2 –61.8	<0.05
Index of osteosclerosis	5.65±6.4	2.4 –32.7	3.8±1.9	1.6 –13.9	<0.001
Adipose tissue %	49.5 ±13.8	1.1 –64.7	18.0±12.4	0.58–55.4	<0.001
Haematopoiesis %	50.5 ±13.9	35.3 –98.9	81.9±12.4	44.6 –99.4	<0.001
Erythropoiesis (E) $\times 10^3$ total/mm^2	31.5 ±5.8	10.0 –41.6	16.9±8.4	6.9 –42.7	<0.001
Granulopoiesis (G) $\times 10^2$ total/mm^2	49.0 ±6.3	37.2 –61.7	30.1±10.0	14.5 –66.1	<0.001
Ratio G/E	1.56±0.3	1.07–2.16	2.0±0.8	0.89–4.2	<0.001
Megakaryopoiesis total/mm^2	15.3 ±3.1	9.8 –22.7	52.3±17.9	15.5 –117.0	<0.001
Density of fibres $\times 10^2$ i/mm^2	16.0 ±5.1	4.1 –19.5	93.5±32.8	16.1 –160.3	<0.001

SD: standard deviation; n.s.: not significant

newly formed intramembranous bone or neo-spongiosa (Fig. 2d). Without regarding these various morphological features which apparently reflect the involving disease process, a survey of basic morphometric data is given in Table 2.

When comparing the data presented in Table 2 for the control specimens with those of OMF patients, wide ranges and a high standard deviation of measurements for bone marrow features are evident. This finding corresponds with the remarkably variable aspect of histopathology as described above (Figs. 1a–d; 2a–d) and may be related to the progress of disease. For this reason an attempt was made to discriminate early and late alterations of the bone marrow by a set of morphometric parameters which are demonstrated in Fig. 3 in comparison with control specimens. However, in this context it should be emphasized that these different stages present a dynamic process and certainly overlaps occur between the arbitrarily chosen cut-off points. In congruence with the complexity of morphometric variables (Table 2) laboratory data in OMF patients also reveal a wide range of values (Table 1). Following discrimination of our 50 patients with OMF according to bone marrow histomorphometry into an early hyperplastic (group-1

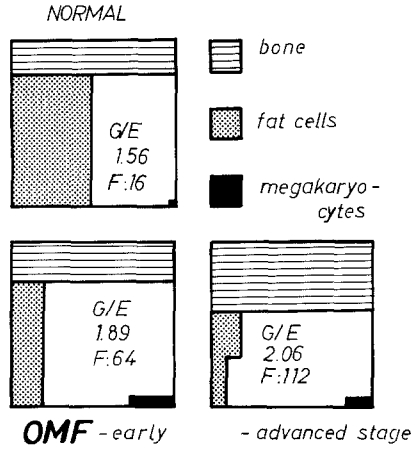
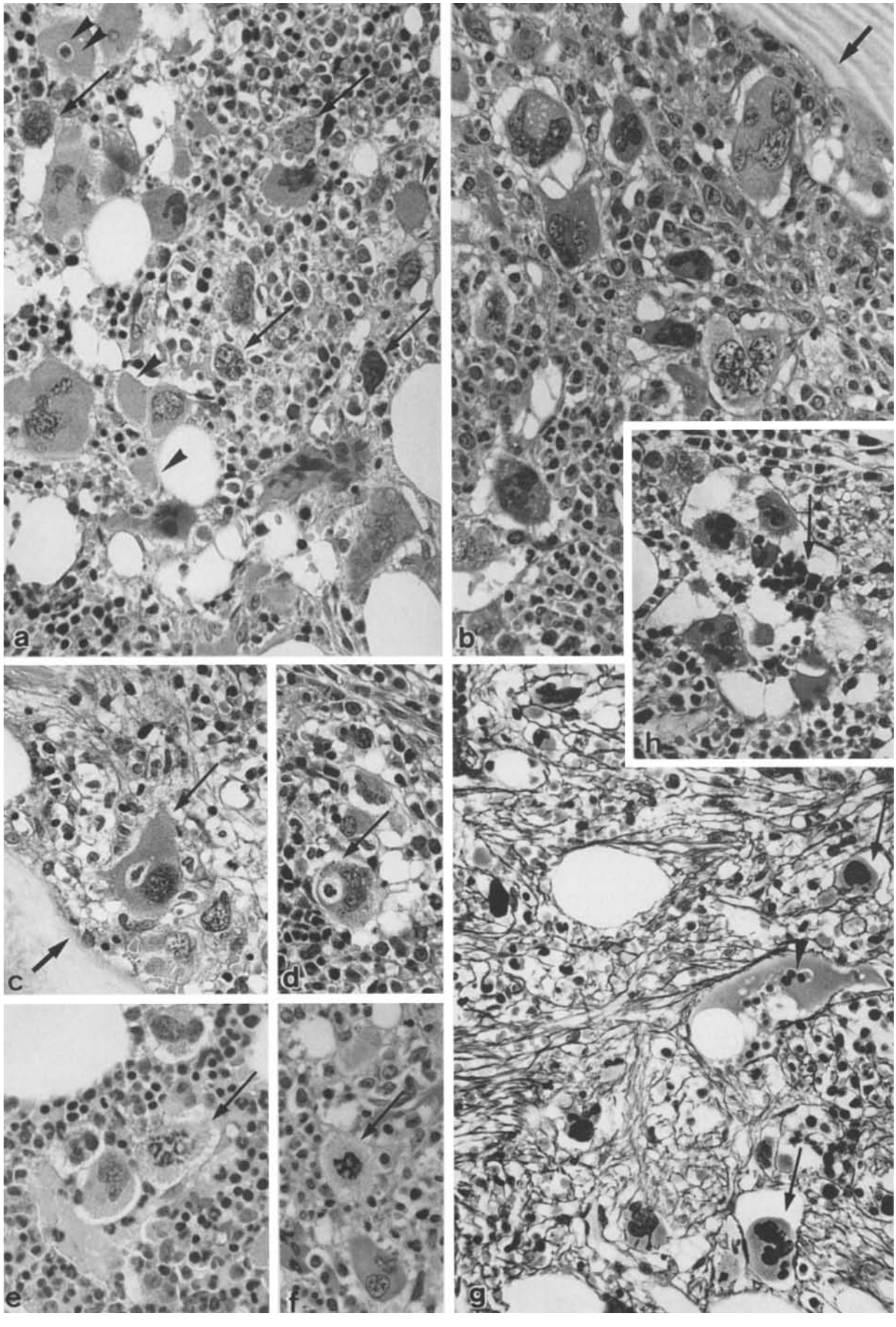


Fig. 3. Schematic presentation of basic morphometric data for one square millimeter biopsy area in early versus advanced stages of primary osteomyelofibrosis/-sclerosis (OMF) in comparison with control specimens. These differences of the two subtypes of patients are illustrated by Figs. 1a, b and 2a, b (group-1 patients) and by Figs. 1c, d and 2c, d (group-2 patients). In this presentation, area of haematopoiesis contains an undetermined number of mast and plasma cells, erythro- and lymphocytes, histiocytic reticulum cells and monocytes. Moreover, at a varying degree this space may be occupied by interstitial oedema and dilated as well as increased vascular structures (see also Fig. 4h, g). G/E = ratio granulo- to erythropoiesis, F = density of fibres $\times 10^2$ i/mm^2

Fig. 4a–g. Megakaryocytes in the bone marrow of patients with OMF. In addition to a pronounced megakaryocytic proliferation there is a conspicuous variety in cell sizes ranging from micro- to giant forms with highly lobulated nuclei **a, b**. Moreover, many anuclear cytoplasmic fragments (**a** *arrow head*) and pycnotic megakaryocytes – naked (*bare*) nuclei may be observed (**a** *arrows*). Infrequently haematopoietic cells are detectable engulfed in the cytoplasm of a megakaryocyte, i.e. emperipolesis (**a** *double arrow* head, **c, d** *arrows*). There is further an atypical dislocation of megakaryocytes towards the endostal generation zone of neutrophilic granulopoiesis (**b, c** *thick arrows*). Mitotic figures are occasionally observable (**e, f** *arrows*). Finally megakaryocytes may be seen in dilated sinuses (**g, h** *arrows*), surrounded by erythrocyte precursor cells (**h** *arrow*) or isolated islets of erythro- and normoblasts (**g** *arrow head*) as intravascular haematopoiesis. **a–g** ×350; **a–f** and **h** PAS reaction, **g** silver impregnation after Gomori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patients, $n=19$) and an advanced fibro-osteosclerotic (group-2 patients, $n=31$) subtype (Fig. 3), the relevant haematological variables showed significant differences in both groups. These differences included haemoglobin level, thrombocyte count, expression of a leucoerythroblastic blood picture and hepatosplenomegaly.

Abnormalities of the megakaryocyte lineage are, together with the fibro-osteosclerotic lesions (Figs. 1c, d; 2c, d), the most conspicuous features of the bone marrow in OMF. Apart from an overall increase (Table 2), a comparison with control specimens discloses numerous pleomorphic and often bizarre looking forms with a dissociation of nuclear-cytoplasmic maturation (Fig. 4a, b). Pleomorphism is represented in particular by an admixture of large to giant megakaryocytes exhibiting extensively segmented, staghorn-like nuclei (Fig. 4a, b) with microforms and many pycnotic or degenerative cells as bare or naked nuclei (Fig. 4a). While the phenomenon of emperipolesis is infrequently encountered (Figs. 4c, d), there is an obvious increase in the counts for megakaryocytes showing mitosis (Figs. 4e, f; Table 3). Irregularity of cell shape, that is to say a tortuous or amoeboid outline (Fig. 4a), is documented by a lower value for the circular deviation factor (Table 3) and also by the frequent occurrence of anuclear cytoplasmic fragments (Table 3; Fig. 4a). A feature that is further a characteristic of advanced stages of OMF are the dilated sinuses containing islets of haematopoiesis, predominantly megakaryocytes surrounded by nucleated erythrocytic precursor cells (Figs. 4h, g). Noticeable are significant differences of certain megakaryocyte features closely associated with an increase in medullary fibrosis (Table 4). These parameters which could be evaluated by histomorphometry may account collectively for a greater expression of pleomorphism or abnormality of this cell lineage concurring with fibrosclerotic changes.

Multiple interactions amongst histomorphometric variables could be observed; however, those between density of fibres and other features of the bone marrow in OMF were most conspicuous (Table 5). Of the clinical variables a number also revealed significant interrelationships, which are mostly not relevant in the context of this study. Most remarkable were the correlations between spleen size and length of preclinical history with density of fibres, degree of osteosclerosis and amount of haematopoiesis (Figs. 5 and 6). As indicator for the progress of disease the duration of characteristic preclinical symptoms was chosen, which could be roughly estimated in all cases in our outpatient clinic. Consideration of the total course of clinically followed disease from diagnosis (or date of bone marrow biopsy) to death was thought to be inaccurate, particularly for the evaluation of (hepato-)splenomegaly, extent of fibrosis or myeloid metaplasia. Because of the obvious influencing of those parameters by therapeutic modalities, such as cytotoxic therapy, radiation and the frequently applied transfusions of packed cells, the length of relevant preclinical history was employed.

Table 3. Histomorphometric features of the megakaryocytic lineage derived from trephine biopsis of the bone marrow at presentation of 50 patients with OMF (see also Table 2) in comparison with control cases

Parameter	Controls means ± SD	OMF means ± SD	Significance (p-value)
Density:			
total/mm^2	15.3 ± 3.1	52.3 ± 17.9	<0.001
nucleated forms %	69.6 ± 4.6	63.8 ± 5.2	<0.001
fragments of cytoplasm %	15.8 ± 3.8	22.3 ± 4.9	<0.001
naked (bare) nuclei %	10.2 ± 2.2	10.8 ± 4.1	n.s.
emperipolesis %	4.5 ± 1.3	2.8 ± 2.1	<0.001
mitosis %	0	0.27 ± 0.5	<0.001
Diameter in µm:			
nucleated forms	23.8 ± 1.8	26.0 ± 4.0	<0.005
fragments of cytoplasm	21.6 ± 2.5	23.1 ± 5.3	n.s.
naked (bare) nuclei	13.5 ± 1.8	14.9 ± 2.4	<0.01
Area: µm^2			
nucleated forms	338.6 ± 47.7	365.5 ± 131.0	n.s.
nuclei	83.6 ± 12.9	87.9 ± 27.4	n.s.
fragments of cytoplasm	273.6 ± 57.4	302.0 ± 143.4	n.s.
naked (bare) nuclei	106.2 ± 22.9	115.6 ± 39.6	n.s.
Circular deviation factor $\times 10^{-2}$			
nucleated megakaryocytes	82.8 ± 3.5	76.4 ± 4.7	<0.001
nuclei	54.1 ± 4.1	51.1 ± 6.23	<0.05
naked (bare) nuclei	70.9 ± 6.9	73.8 ± 4.7	n.s.
Ratio:			
nucleus/cytoplasm area	0.25 ± 0.02	0.26 ± 0.03	n.s.

SD: standard deviation; n.s.: not significant

Table 4. Significant differences of certain histomorphometric features of megakaryocytes concurring with an approximately twofold increase in medullary fibrosis, i.e. between early hyperplastic and advanced fibro-osteosclerotic stages of OMF. These features may account for a more conspicuous pleomorphism or abnormality in the appearance of this cell line during evolution of myelofibrosis

Histomorphometric variables	Early hyperplastic ($n=19$)	Late fibro-osteosclerotic ($n=31$)	Level of significance (p-value)
Density of fibres $\times 10^2$ i/mm^2	63.7±20.1	111.8±24.7	<0.01
Megakaryocyte			
– size µm^2	429.2±144	326 ±104	<0.01
– circular deviation factor $\times 10^{-2}$	78.2±4.2	75.3±4.6	<0.05
Nucleus			
– size µm^2	103±31	79±20	<0.01
Naked (bare) nuclei			
– frequency %	10.3±3	12.2±3	<0.01
– size µm^2	134 ±39	107 ±35	<0.01
– circular deviation factor $\times 10^{-2}$	75.4±4.4	72.8±4.5	<0.05

The p-values were gained by applying the t-test

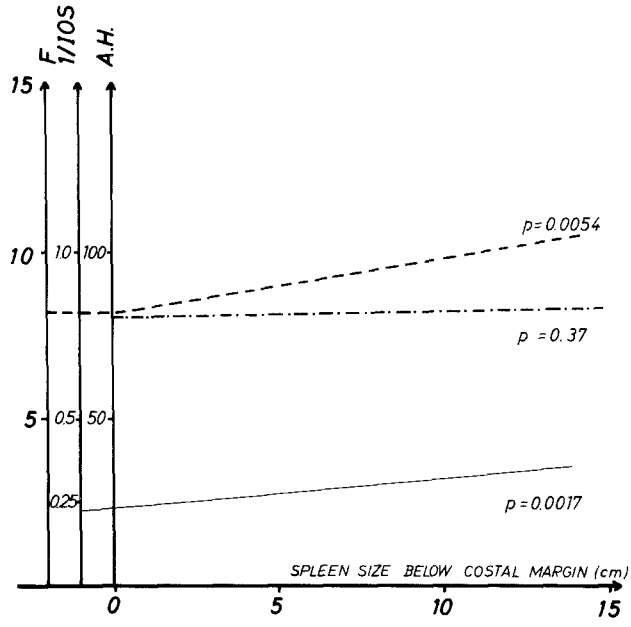

Fig. 5. Significant correlations between splenomegaly (cm below costal margin) and degree of osteosclerosis (1/IOS) as well as density of fibres ($\times 10^2$ i/mm^2). Note the failure to show a significant relationship between amount of haematopoiesis within the bone marrow and spleen size ($p=0.37$). –·–·– A.H.: Area of haematopoiesis (%); —— I/IOS; Index of osteosclerosis; – – – F: Density of fibres (i/mm^2) $\times 10^2$

Table 5. Significant correlations ($p<0.05$) among various histomorphometric features of the bone marrow in 50 patients with OMF. All other variables tested were not significant

Histomorphometric variables		Level of significance (p-value)
	versus	
Density of fibres $\times 10^2$ i/mm^2	erythropoiesis $\times 10^2$/mm^2	–0.0018
	granulopoiesis $\times 10^2$/mm^2	–0.001
	naked (bare) nuclei/mm^2	+0.0087
	megakaryocyte size µm^2	–0.015
	naked nuclei size µm^2	–0.0015
	circular deviation of megakaryocytes – factor	–0.0049
	1/osteosclerosis index (IOS)	+0.0015
Megakaryocyte size µm^2	circular deviation of nuclei – factor	–0.0017
Degree of osteosclerosis 1/IOS	erythropoiesis $\times 10^2$/mm^2	–0.026
	granulopoiesis $\times 10^2$/mm^2	–0.0072

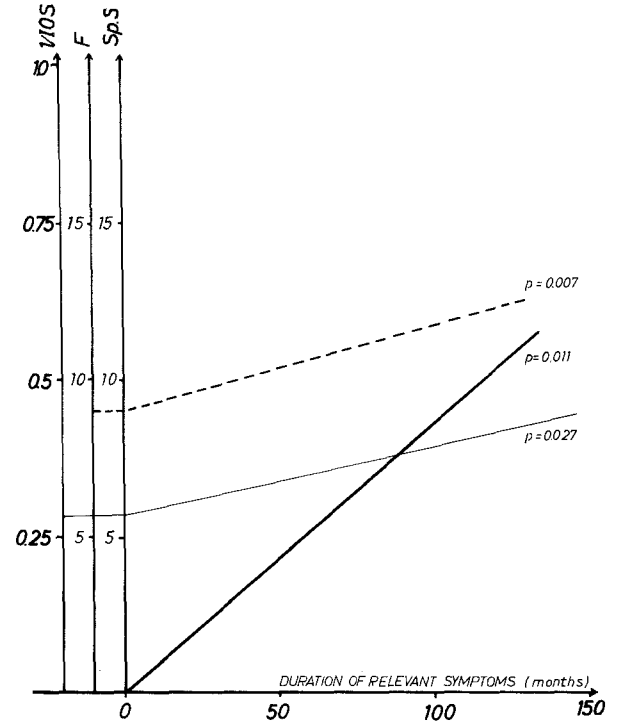

Fig. 6. Significant correlations between duration of relevant prediagnostic symptoms and degree of osteosclerosis (1/IOS), density of fibres ($\times 10^2$ i/mm^2) and splenomegaly (cm below costal margin). —— Sp.S.: Spleen size (cm); – – – F: Density of fibres (i/mm^2) $\times 10^2$; —— I/IOS: Index of osteosclerosis

Discussion

Our results show a significant correlation between duration of preclinical history (progress of disease at presentation of patients) and density of fibres, spleen size and degree of osteosclerosis (Fig. 6). This implies that in OMF the evolution of the disease features involves a stage-like process (Lewis 1985). At the onset this process may be described as a myeloproliferative disease without increase in reticulin fibres or remodelling of trabecular bone accompanied by a minimal (hepato-) splenomegaly and non-characteristic clinical findings (slight anaemia). However, the full-blown clinical picture of OMF from laboratory data showed some degree of fibro-osteosclerosis in the bone marrow as well as an enlargement of spleen and liver and a leucoerythroblastic reaction (Geary 1985). Both stages in the development of OMF could be distinguished by their basic histomorphometric parameters (Fig. 3). In this regard previous descriptions of bone marrow histology obtained from sequential biopsies at variable intervals during the lengthy course of disease have been confirmed and extended (Lohmann and Beckman 1983; Frisch et al. 1984; Burkhardt et al. 1984, 1986; Georgii et al. 1984a, b; Thiele et al. 1988).

The failure to document a progression of medullary fibrosis and a correlation between duration of disease, extent of myelofibrosis and spleen weight or size by some authors (Bentley and Herman 1979; Wolf and Neiman 1985) may be explained by considering the patients recruited for these studies as well as the methods applied for assessment of these data. In five of the 35 patients reported by Wolf and Neiman (1985) the diagnosis of AMM was made during the course of pre-existing P. vera, which is not in accordance with our rigid definition of OMF (see Materials and methods). In at least three of the 17 patients described by Bentley and Herman (1979) CML complicated by myelofibrosis could not be excluded with certainty. Histological parameters, but particularly the content of fibres which were later correlated with spleen size or weight were either roughly estimated (Wolf and Neiman 1985) or determined by a digital image processing technique (Bentley and Herman 1979). In both studies a possible interference of these changes with therapeutic modalities was not considered, although a variety of regimens had been employed. Of the 35 patients enrolled by Wolf and Neiman (1985) 14 cases had repeated transfusions, five had been treated with cytostatics (busulfan) in an attempt to control hypersplenism, and two received radioactive phosphorus for thrombosis. In the second study pertinent to this problem (Bentley and Herman 1979), there was no precise information about treatment.

Noteworthy is our finding that amount of haematopoiesis (neutrophilic granulocytes, nucleated erythrocytic precursors and megakaryocytes) determined by morphometry on large-sized trephine biopsies did not disclose any significant correlations with splenomegaly (Fig. 5). This result suggests that degree of splenic myeloid metaplasia is not necessarily dependent on medullary cellularity; extramedullary haematopoiesis may present an autonomous process and not compensation for a failing fibrotic and osteosclerotic bone marrow. In this regard our findings substitute the assumptions made by several authors (without employment of morphometry or statistical evaluation) that there is no predictable relationship between bone marrow morphology and degree of splenic extramedullary haematopoiesis, in particular splenic size and weight (Ward and Block 1971; Pitcock et al. 1982; Wolf and Neiman 1985). It remains debatable whether extramedullary haematopoiesis may be explained by a myelostimulating factor (Dameshek 1951) resulting in "seeding" or "re-awakening" of a monoclonal population of stem cells or their progeny at potential extramedullary sites for haematopoiesis, presumably originating from medullary sources (Linman and Bethell 1957; Ward and Block 1971; Gilbert 1973; Pitcock et al. 1982). In recent years this reactivation of the haematopoietic potential at sites of embryonal haematopoiesis has been contested, since it has been demonstrated that the human fetal spleen is not a haematopoietic organ (Wolf et al. 1983). A hypothesis was proposed connecting the prominent intravascular haematopoiesis in the bone marrow of patients with OMF (Figs. 4h, g) or AMM with a decompensation of splenic filtration of these cells (Wolf and Neiman 1987).

The pathogenesis of fibro-osteosclerotic changes characterizing OMF is not well understood. There is general agreement that myelofibrosis is related to an increase in marrow collagen accumulation and that the source of the collagen synthesis is in the fibroblasts. Recent cytogenetic studies of fibroblasts in a patient with OMF and trisomy 8 revealed that myelofibrosis represents a secondary, non-neoplastic reaction to the clonal proliferation of haematopoietic stems cells (Greenberg et al. 1987) thus confirming earlier findings (Jacobson et al. 1978). Several reports have postulated that reticulin and collagen depositions may be mediated by an immunological mechanism (Lewis and Pegrum 1978; Caligaris-Cappio et al. 1981; Rozman

et al. 1983; Rondeau et al. 1983), however, the appearance of immune complexes in OMF may be a secondary rather than a primary phenomenon (Gordon et al. 1981). It is reasonable to assume that the intramedullary release of platelet-derived factors by the abnormal megakaryopoiesis (Ross and Vogel 1978; Kaplan et al. 1979; Castro-Malaspina et al. 1981; Burstein et al. 1984) triggers the proliferation of fibroblasts and collagen synthesis: growth factor stimulates this process and factor 4 inhibits the activation of the enzyme collagenase, conversely leading to myelofibrosis (Castro-Malaspina 1984). As was shown by our study (Tables 4 and 5), there is a significant correlation between density of reticulin and collagen fibres with certain megakaryocyte features evaluable by morphometry (size, naked-pycnotic nuclei, circular deviation). These data suggest an increasingly abnormal or at least pleomorphic appearance of this cell line associated with an evolving medullary fibrosis. Principally, however at a qualitatively lower level, these alterations of the megakaryopoiesis are already expressed at the very early (hyperplastic) stages without any increase in reticulin fibre content in comparison with the control specimens (Figs. 1a, 2a). For this reason they are thought to precede myelofibrosis. In this context it should be mentioned that in OMF the absolute number of megakaryocytes per square millimeter bone marrow area alone does not seem to be the salient point for the development of myelofibrosis, because we were not able to calculate significant correlations between density of fibres or osteosclerosis (IOS) with this variable. The failure to document some interactions between those variables should be expected when taking into account the so-called early hyperplastic stages of OMF (group-1 patients, Fig. 3). These cases did not display relevant fibro-osteosclerotic changes of the bone marrow, but a pronounced growth of megakaryocytes (Figs. 1a, b; 2a, b). Increase in bone marrow matrix and abnormal megakaryocytic proliferation is a prerequisite for osteosclerotic changes, as was again demonstrated by our morphometric measurements (Table 5). Calcitriol (1,25 dihydroxy vitamin D3), the active metabolite of vitamin D3, antagonizes collagen synthesis by suppression of megakaryocyte proliferation and stimulation of monocytes which produce collagenase. A deficiency of this factor may collectively contribute not only to an abnormal increase in medullary fibrosis (McCarthy 1985), but may finally generate osteosclerotic alterations of the trabecular bone. In consideration of these complex mechanisms a new approach for therapeutic trials in myelofibrosis has recently been proposed (Arlet et al. 1984; Fruchtman 1984; Petrini et al. 1986; Richard et al. 1986; McKinley et al. 1987; Duncombe et al. 1987; Manoharan et al. 1988).

In summary, our histomorphometric study on trephine biopsies biopsies of the bone marrow in OMF reveals that there is a fibrosclerotic process in the evolution of disease features related to an abnormal megakaryopoiesis and associated with certain clinical data. However, the degree of myeloid metaplasia is thought to represent an autonomous or neoplastic process and not a compensation for a failing progressively fibrotic and osteosclerotic bone marrow.

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