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Myeloid Dysplasia (MD): a Hematological Disorder Preceding Acute and Chronic Myeloid Leukemia

A Morphological Study on Sequential Core Biopsies of the Bone Marrow in 27 Patients *

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Summary. Light- and electron microscopic appearances of core biopsies of the bone marrow in 27 selected patients out of about 195 cases with a clinically suspected preleukemic syndrome. The correct diagnosis of "preleukemia" was established retrospectively by sequential biopsies of the iliac crest or autopsy in those patients who developed overt leukemia in periods ranging from 2 to 36 months. As a final diagnosis chronic myelogenous leukemia (CML) was established in 10, with accompanying blast crisis in 6 and acute non-lymphocytic leukemia (ANLL) in 11 cases. Histomorphology of the resin embedded cores of bone marrow showed hypercellularity in 21 specimens, a hypocellular marrow in 5 and a normal bone marrow in 1 case. There was also a conspicuous macrocytic or megaloblastoid maturation of erythropoiesis with frequent sideroblasts. Ultrastructural abnormalities included atypical nuclear clefts, dense iron deposits in the mitochondrial matrix and an increase of ferritin uptake. Neutrophilic granulopoiesis showed a shift to the left and often a remarkable aberration of nuclear segmentation consistent with a pseudo-Pelger-Huët anomaly. Electron microscopy displayed atypias of granulogenesis in comparison with maturation and segmentation of the nuclei, abnormal nuclear loops and blebs and very conspicuous nuclear fibrillar appendages (so called Nebenkerne). There was also an increase in eosinophilic granulocytes, monocytic elements, edema and a remarkable perivascular plasmacytosis of the myeloid stroma. Our results suggest that characteristic morphological features of the bone marrow exist before onset of overt, acute and chronic leukemia. These alterations are identical in CML and ANLL and are the morphological substrate of a maturation defect of hematopoiesis which precedes the establishment of the leukemic clone. The clinical term preleukemia should be replaced by myeloid dysplasia (MD), thus indicating transformation into overt leukemia in only a certain propor-

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tion of patients (only 27 of 195 clinically suspected patients who displayed an identical histopathology of MD in the bone marrow in 93 cases to date) and has to include ANLL as well as CML.

Key words: Preleukemia – Myeloid dysplasia – Bone marrow biopsy – Chronic myeloid leukemia – Acute non-lymphocytic leukemia.

Introduction

The term preleukemia was coined by Block et al. (1953) to characterize lesions of the bone marrow which precede overt acute non-lymphocytic leukemia (ANLL). In recent years, abundant data concerning the clinical, laboratory, and morphological manifestations of the preleukemic syndrome have been gathered (reviews by Linman and Bagby 1976, 1978; Heimpel 1976; Jouet et al. 1978; Majolino et al. 1978; Pierre 1978; Fischer and Schaefer 1979). These investigations were mostly stimulated by the discussion of a clear cut definition of the term preleukemia encompassing various syndromes which were already known to clinicians to be potentially leukemic disorders. This applies particularly to aplastic anemia, refractory megaloblastic or sideroblastic anemia (Dreyfus 1976) or acquired lesions frequently leading to acute leukemia following radiation, drug or chemical exposure (reviews by Gross et al. 1973; Pierre 1974). In spite of the many predominantly clinical studies of preleukemic disorders, designated preleukemic states or syndromes, early leukemia or even oligoblastic or smoldering leukemias (review by Bessis and Brecher 1976), the histopathology of this entity still remains ill defined. This is due to several factors: limitation of histopathology to aspirates of the bone marrow in most studies (Saarni and Linman 1973; Schmalzl et al. 1978; Linman and Bagby 1978), the concept that preleukemia precedes ANLL only, and the scarcity of ultrastructural investigations. This study attempts to demonstrate that the characteristic features of the bone marrow can be found in prephases of *acute* and *chronic* myelogenous leukemias.

Material and Methods

Patients. Twenty-seven patients (13 female, 14 male, aged between 35–77 years) from our files of about 195 cases of clinically and/or histologically suspected preleukemic syndromes will be described in this investigation. These 27 patients were selected retrospectively, since they later turned out to evolve into overt acute and chronic myeloid leukemia (11 and 16 cases respectively). 6 cases had a blastic crisis. These diagnoses were established by clinical data (Tables 1 and 2), sequential core biopsies (up to 5) of the bone marrow (Tables 3 and 4), and cytogenetic analysis of the karyotypes as reported elsewhere (Thiele et al. 1979a). In 5 of these cases an autopsy was performed in our laboratory (Table 5). The subgroups (CGL and CMGM) of chronic myelogenous leukemia (CML) were classified according to Georgii (1979). The preleukemic period or the prodromal phase of leukemia manifestation as observed by clinical and histomorphological follow up studies lasted at least 2 months till over 3 years and is given in Table 6 separately for AML/ANLL and CML/CMGM respectively. No patient was receiving any cytostatic therapy or corticosteroids at the time of the first biopsy, i.e., the preleukemic stage; for further clinical data see Thiele et al. (1981).

Table 1. Comparison of some clinical and morphological findings in 16 patients who developed chronic myeloid leukemia, at the time of the first and last biopsy of the bone marrow. CML: chronic myeloid leukemia, CGL: chronic granulocytic leukemia, CMGM: chronic megakaryocytic-granulocytic myelosis; + hyper, − hypocellular bone marrow

Patients		Age years	Sex	First biopsy of the bone marrow			Final biopsy (or necropsy) of the bone marrow		
				Clinical diagnosis	Hepato-splenomegaly	Cellularity	Clinical diagnosis	Histological diagnosis	Cytostatic therapy
1.	W.W.	49	M	Vit. B_{12} resistant anemia	−	+	hyperplastic panmyclopathy with transition into CGL	CGL	−
2.	R.M.	68	F	leukemia?	−	+	myeloproliferative syndrome	CGL	+
1.	T.L.	73	F	hyperplastic panmyelopathy	++	+	osteomyelofibrosis	CMGM	−
2.	J.G.	70	F	splenomegaly, thrombosis	++	+	CML	CMGM	−
3.	S.P.	72	M	myeloproliferative syndrome?	+	+	CML	CMGM	−
4.	O.E.	62	M	anemia	++	+	osteomyelofibrosis	CMGM	−
5.	S.A.	60	M	anemia	++	+	early stage of CML	CMGM	−
6.	G.B.	58	M	anemia	++	+	smouldering leukemia	CMGM	−
7.	H.K.	38	F	anemia	−	+	myelofibrosis?	CMGM	−
8.	B.F.	63	M	anemia	+	+	early CML	CMGM	−
1.	H.H.	68	M	myeloproliferative syndrome?	++	+	CGL in blastic crisis	CGL in blastic crisis	+
2.	K.H.	60	F	pancytopenia	+	+	CML in blastic crisis	CGL in blastic crisis	+
3.	S.W.	77	M	pancytopenia	+	+	CML in blastic crisis	CGL in blastic crisis	+
1.	A.K.H.	68	M	sideroblastic anemia	+	+	CML in blastic crisis	CMGM in blastic crisis	−
2.	S.O.	70	M	leukopenia	−	+	myeloproliferative syndrome	CMGM in blastic crisis	−
3.	V.L.	66	M	aplastic syndrome	−	−	CML in blastic crisis	CMGM in blastic crisis	+

Table 2. Comparison of some clinical and morphological findings in 11 patients who evolved acute myeloid leukemia (AML), at the time of the first and last biopsy of the bone marrow; + hyper, − hypocellular bone marrow

Patients	Age years	Sex	First biopsy of the bone marrow			Final Biopsy (or necropsy) of the bone marrow		
			Clinical diagnosis	Hepato-spleno-megaly	Cellu-larity	Clinical diagnosis	Histo-logical diag-nosis	Cyto-static therapy
1. K. H.	66	F	anemia	+	normal	panmyelopathy – preleukemia	AML	−
2. M. K.	52	F	pancytopenia	+	+	AML	AML	−
3. M. O.	77	M	anemia, hypersensitivity disease	+	−	AML	AML	+
4. W. O.	48	F	panmyelopathy	+	+	AML	AML	+
5. F. E.	35	F	panmyelophthisis	−	+	aplastic syndrome with transition into AML	AML	+
6. S. K.	59	F	pancytopenia	−	+	AML	AML	+
7. B. H.	60	M	pancytopenia	+	−	oligoblastic myeloid leukemia	AML	+
8. K. F.	71	M	pancytopenia	−	+	myeloproliferative syndrome	AML	+
9. K. D.	47	F	megaloblastic anemia	−	+	CML in blastic crisis	AML	+
10. W. L.	72	F	pernicious anemia	+	+	AML	AML	+
11. G. M.	67	F	anemia	+	+	AML	AML	+

Methods. Core biopsies of the bone marrow were obtained either by the technique of myelotomy from the anterior iliac crest (Burkhardt 1966, 1971) or by Jamshidi's method from the posterior crest (Jamshidi and Swaim 1971). Histological processings of resin embedding with mixtures of acrylates to perform semithin sections of 1–3 μ and hematological stainings have been described by Vykoupil et al. (1976) in more detail. The technical procedures applied in electron microscopy of the 3 patients investigated are recorded elsewhere (Thiele et al. 1977). Two of those patients studied by electron microscopy later evolved into AML and one into CML.

Results

The first biopsies of the sequential corings of the bone marrow (see also Tables 3 and 4) present the clinical and morphological precursors of overt leukemia,. The clinical histories preceding this diagnostic trephine biopsy of the iliac crest include general malaise, anemia and frequently pancytopenia (panmyeloph-

Table 3. Schematic drawing of followed course of patients with CML (CGL: chronic granulocytic leukemia; CMGM: chronic megakaryocytic–granulocytic myelosis). ↓: core biopsy with myeloid dysplasia, ↕: core biopsy with overt leukemia, Ph'+: Philadelphia chromosome shown in direct preparations of the bone marrow, Ph'–: no Philadelphia chromosome; asteriks indicate autopsied patients

Patients	Age years	Sex	Period of clinical and morphological investigation from preleukemic state to overt leukemia	Final diagnosis
1. W.W.	49	M		CML
2. R.M.	68	F	Ph'+	(CGL)
1. T.L.	73	F		
2. J.G.	70	F		
3. S.P.	72	M		
4. O.E.	62	M	further 3 years	CML
5. S.A.	60	M	Ph'+	(CMGM)
6. G.B.	58	M		
7. H.K.	38	F		
8. B.F.	63	M		
1. H.H.	68	M	Ph'+ further 2 years	CML
2.* K.H.	60	F	Ph'+	(CGL)
3. S.W.	77	M	Ph'+	in blastic crisis
1.* A.K.H.	68	M	Ph'+	CML
2. S.O.	70	M	Ph'+	(CMGM)
3. V.L.	66	M	Ph'–	in blastic crisis

12 6 0 6 12 18 24 months

specific clinical history — first of bone biopsy marrow — followed course

Table 4. Schematic drawing of followed course of patients with ANLL; asteriks indicate autopsied patients. Legends of abbreviations and signs are shown in Table 3

Patients	Age years	Sex	Period of clinical and morphological investigation from preleukemic state to overt leukemia	Final diagnosis
1. K.H.	66	F		
2.*M.K.	52	F		
3. M.O.	77	M		
4.*W.O.	48	F	Ph'–	
5. F.E.	35	F		ANLL
6. S.K.	59	F		(AML)
7.*B.H.	60	M		
8. K.F.	71	M	Ph'–	
9. K.D.	47	F	Ph'–	
10. W.L.	72	F		
11. G.M.	67	F		

12 6 0 6 12 18 24 30 months

specific clinical history | first of biopsy bone marrow | followed course

thisis, panmyelopathy) of a variable degree. Minimal to moderate hepatosplenomegaly was present in several cases (Tables 1 and 2).

Surveys of the bone marrow show hypercellularity in 23 patients (Figs. 1a, b; 5a–c), in contrast to one normo- and three hypocellular specimens (Fig. 2b). Regardless of the final diagnosis of chronic or acute leukemia (CML versus AML/ANLL), it is most noticeable that the alterations of the bone marrow are identical at the time of the first biopsy (in the preleukemic state). Table 7 is a synopsis of the light and electron micrsocopic findings obtained from the earliest of those sequential bone marrow examinations. They are described in more detail below:

Erythropoiesis

Light microscopy displays a conspicuous macrocytic generally megaloblastoid differentiation in all cases, covering extensive islets. These erythropoietic areas

Table 5. Relevant autopsy findings in 5 patients who developed chronic and acute myeloid leukemia as a sequel of myeloid dysplasia (so called preleukemia, compare with Table 3 and 4)

Patients	Age years	Sex	Bone marrow		Liver		Spleen		Lymph nodes	Cause of death
			vertebra	femur	weight (g)	infiltrates	weight (g)	infiltrates	infiltrates	
1. K. H.	60	F	CGL in blastic crisis	blastic transformation	2,040	+++	280	+++	+	pneumonia (Klebsiella pneumoniae)
2. A.K.H.	68	M	CMGM in blastic crisis	myeloid metaplasia	4,100	+++	1,620	+++	+++	pneumonia
3. M. K.	52	F	AML	blastic transformation	2,070	+++	940	+++	+	cerebral hemorrhage
4. W. O.	48	F	AML	blastic transformation	1,800	+++	880	+++	–	pneumonia
5. B. H.	60	M	AML	myeloid metaplasia with myeloblasts	3,450	++	250	++	–	sepsis

Table 6. Period of latency preceding evolution of acute and chronic myeloid leukemia from a prodromal phase or so called preleukemia. These data are mainly derived from clinical histories, laboratory parameters and finally histomorphology of sequential trephine biopsies of the bone marrow

Type of leukemia	No.	Range (months)	Median values (months)	Mean	
AML (ANLL)	11	2–30	6	9	$p<0.05$
CML (CGL and CMGM)	16	6–36	13	15	

are frequently dislocated from the central marrow space towards the osseous trabecula (Figs. 1b, c; 6c). There are many sideroblasts with scattered and finely granular deposits of iron in the cytoplasm, but only rarely are ring sideroblasts seen.

Electron microscopy confirms the large numbers of erythrocytic precursor cells with extensive clusters of erythroblasts and early normoblasts (Fig. 2a). In addition there are also erythroblasts with megaloblastoid features of the nucleus (finely dispersed chromatin, prominent and extended nucleoli and many polyribosomes in the cytoplasm (Fig. 3a)). These cells and the more mature erythroblasts exhibit an increased uptake of ferritin as demonstrated by numerous endocytic invaginations and vesicles (so called rhopheocytosis, Fig. 3b).

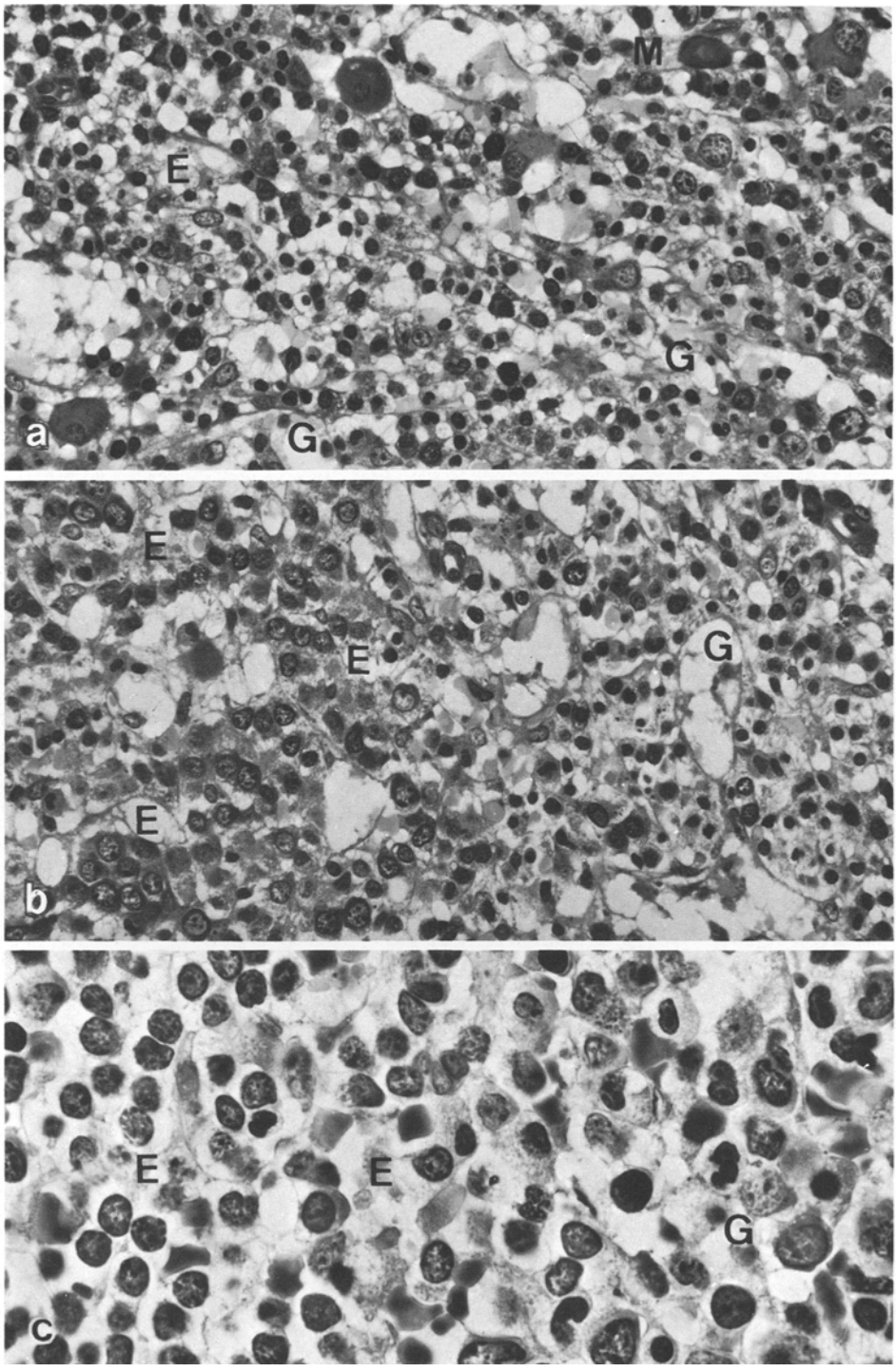


Fig. 1 a–c. Light microscopy of the bone marrow in myeloid dysplasia. **a** Survey displaying a hypercellular marrow (so called "hyperplastic panmyelopathy") with increase of all cell lineages. **b** Erythropoiesis with extensive islets and partially macrocytic differentiation. **c** Macrocytic maturation of erythropoiesis. *E*, erythropoiesis; *G*, granulopoiesis; *M*, megakaryocytes. **a** and **b** ×370; **c** ×980

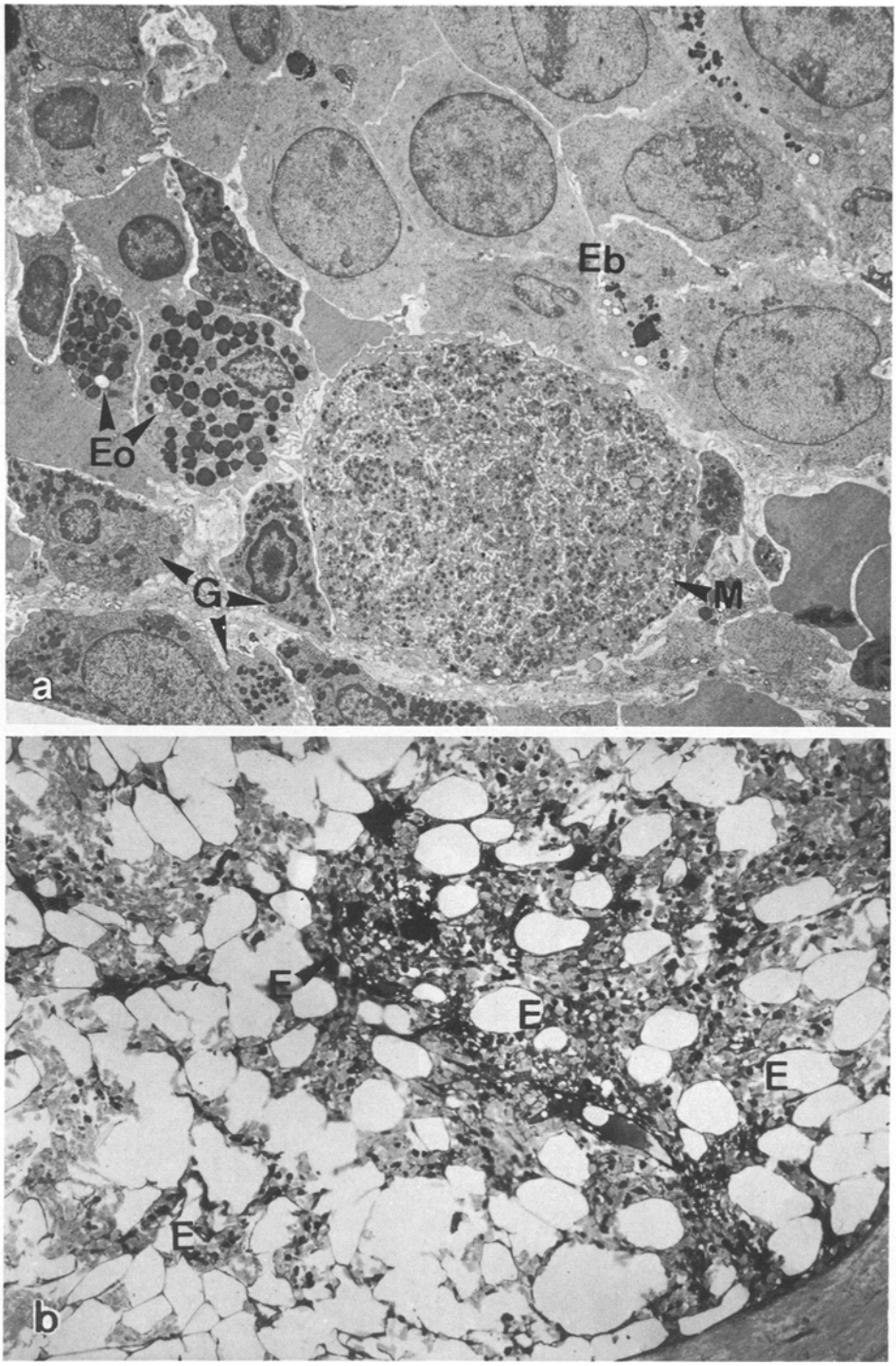


Fig. 2. **a** Electron microscopy of myeloid dysplasia showing large cluster of early erythroblasts (*Eb*) surrounded by granulopoiesis (*G*) with eosinophilic metamyelocytes (*Eo*) and part of a large megakaryocyte (*M*) with extensive demarcation membrane system. **b** Light microscopy of hypocellular marrow ("hypoplastic panmyelopathy") with small islets of macrocytic erythropoiesis (*E*) and a reduction of granulopoiesis. **a** × 5,500; **b** × 170

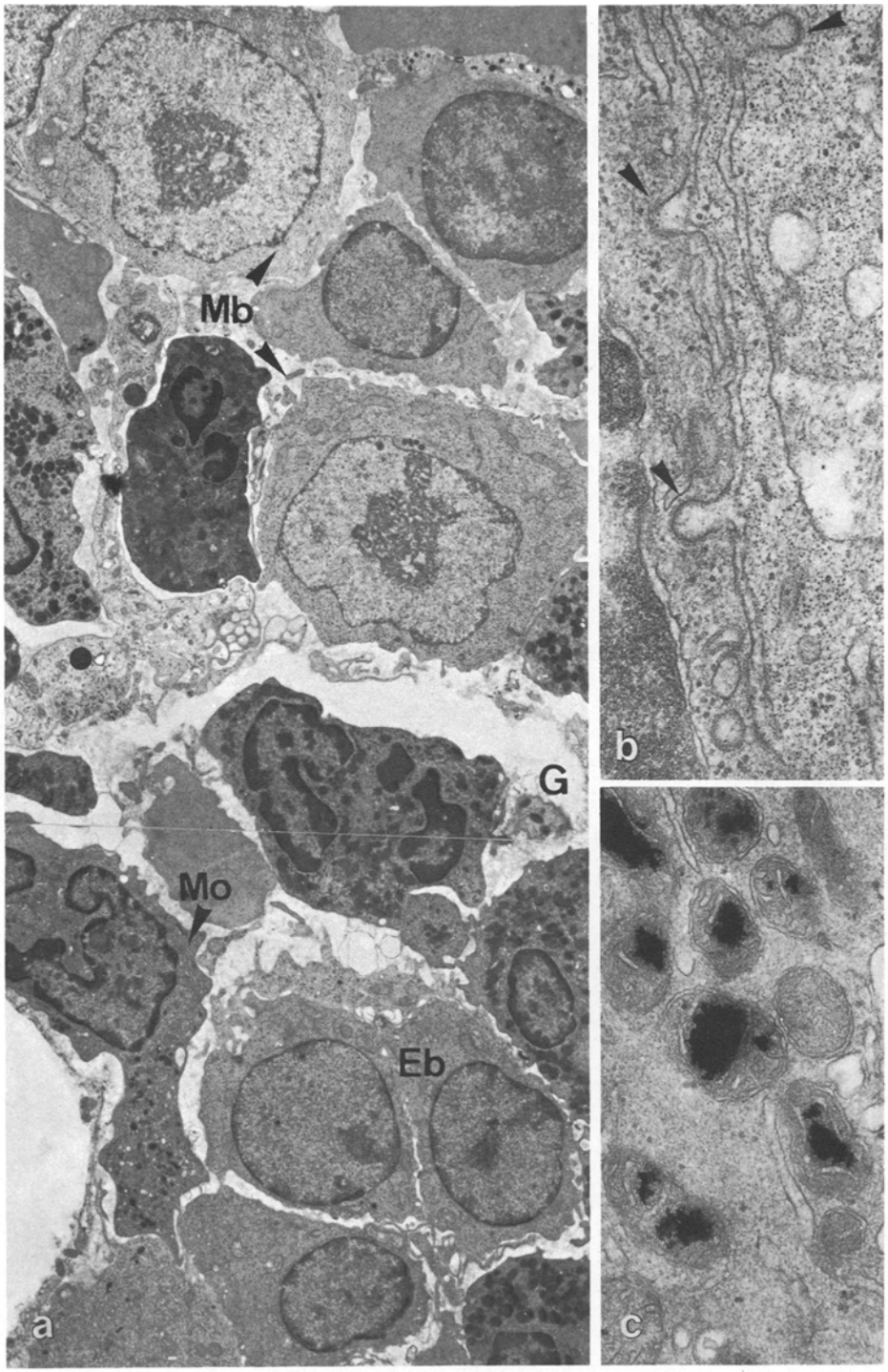


Fig. 3a–c. Ultrastructure of erythropoiesis in myeloid metaplasia. **a** Megaloblastoid features (*Mb*) of erythroblasts (*Eb*) in addition to granulopoiesis (*G*) and part of a mature monocyte (*Mo*). **b** Numerous caveolae with a fuzzy coat (*arrow heads*) suggest increased endocytic activity in these erythroblasts. Ferritin particles are observed within the caveolae, some vesicles and the cytoplasmic ground substance. **c** Mitochondria with coarse and electron dense deposits, presumably iron in their matrix. **a** ×5,000; **b** and **c** ×45,000

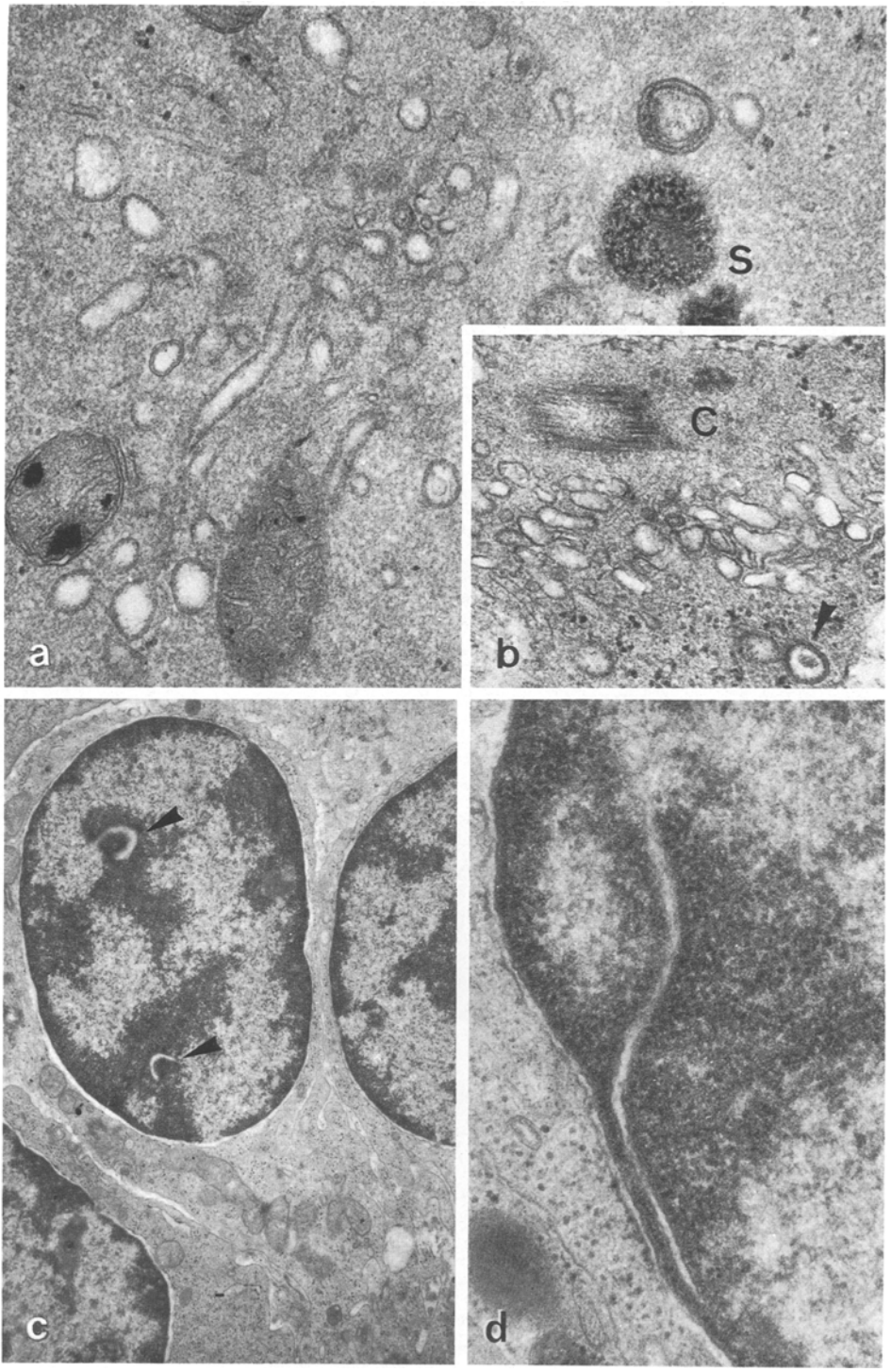


Fig. 4a–d. Ultrastructure of erythropoiesis in myeloid dysplasia. **a** Area with many vesicles and tubules with a bristle coating, possibly due to ferritin transport to the saccules of the Golgi apparatus nearby. Further a mitochondrium with ferruginous micelles, which constitute dense blocks in the interior of the matrix (compare with Fig. 3c) and siderosomes (*S*). **b** Golgi apparatus of an erythroblast surrounding a centriole (*C*) with stack of saccules and a few vesicles with a fuzzy coat (*arrow head*). **c** Nucleus of late erythroblast with two clefts of the chromatin in en-face view (*arrow heads*) associated with heterochromatin regions. **d** Nuclear cleft transversing the peripheral portion of heterochromatin. **a**, **b** and **d** ×45,000; **c** ×15,000

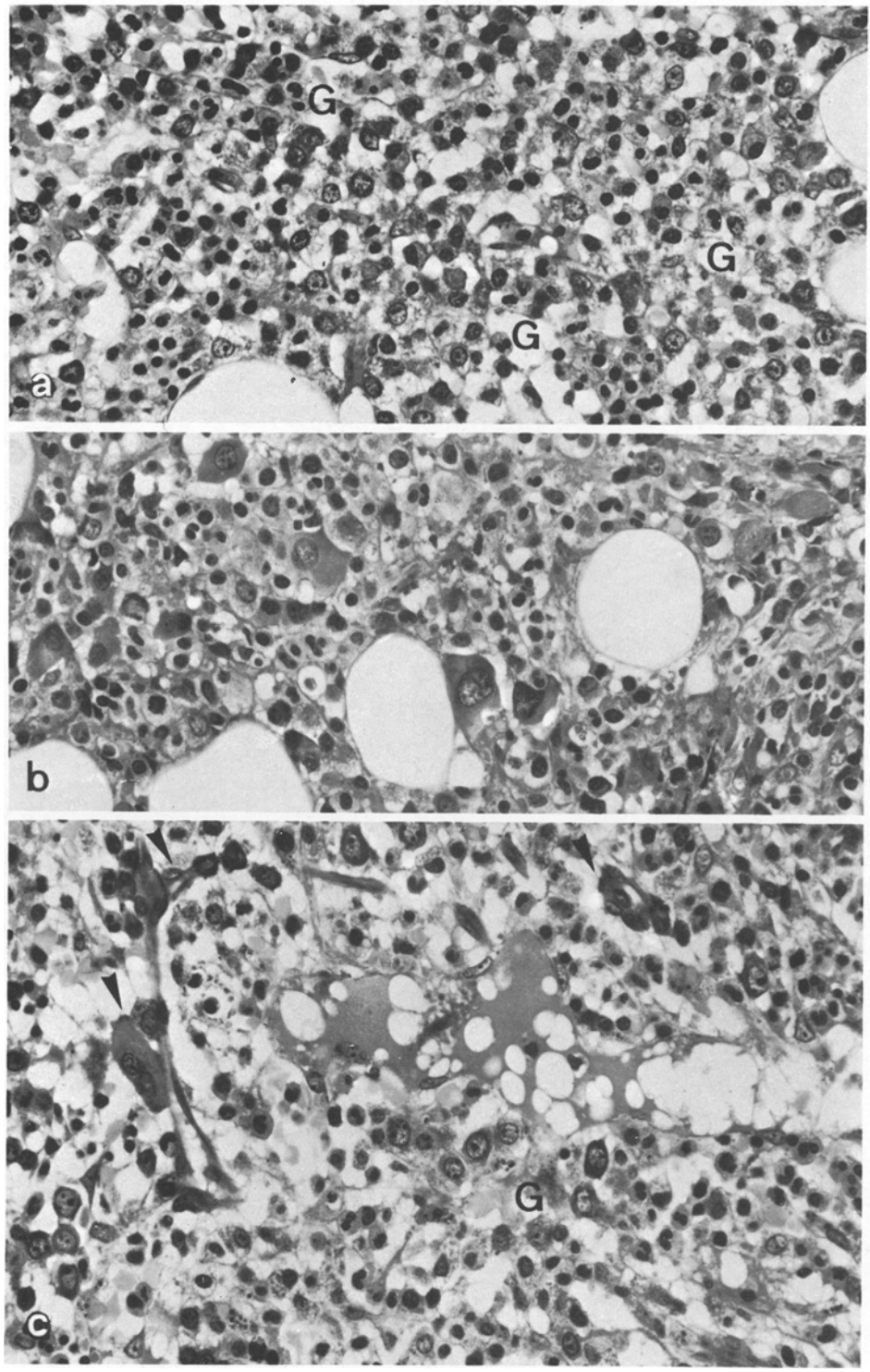


Fig. 5a–c. Light microscopy of granulopoiesis and myeloid stroma in myeloid dysplasia. **a** Survey with increase of neutrophilic granulocytes and precursor forms (myeloblasts-promyelocytes, *G*). **b** Megakaryopoiesis with polymorphism of megakaryocytes and scleredema of the myeloid stroma. **c** Small vessels surrounded by plasma cells (perivascular plasmacytosis) and a megakaryocyte (*arrow heads*). Edema of the myeloid stroma with increase of myelocytes (*G*). **a–c** × 370

Table 7. Myeloid dysplasia (MD). Synopsis of light- and electron microscopical findings in core biopsies of the bone marrow with hypercellular as well as in the rare cases of hypocellular marrow, both finally leading to chronic and acute myeloid leukemia

Cell lines	Light microscopy		Electron microscopy
	Quantitatively	Qualitatively	
Erythro-poiesis	hyperplasia: diffuse extension towards osseous trabecula	disturbance of maturation: macrocytic-megaloblastoid differentiation, delay of maturation, granular sideroblasts	megaloblastoid features with abnormal clefts and blebs of nuclei; siderin deposits in mitochondria, increased rhopheocytosis.
Neutro-phil granulo-poiesis	hyperplasia: proliferation mostly localized hypoplasia: dislocation of immature cells into marrow centers	arrest of maturation: dissociation of nuclear-cytoplasmic development, pseudo-Pelger-Huët anomaly	atypical nuclear loops and blebs, fibrillar appendages of the nuclei; frequent hypergranularity of the cytoplasm, smooth membrane complexes
Mega-karyo-poiesis	hyperplasia: perisinusoidal and extended towards peritrabecular zone of neutrophilic granulopoiesis	abnormalities of differentiation: increase with polymorphous frequently atypical microforms	small megakaryocytes, dissociation of nuclear maturation and development of demarcation system. Large to giant thrombocytes, pleomorphy of granulation, preservation of the Golgi apparatus.

There are many abnormal sideroblasts which show not only large clumps of ferritin in the cytoplasm but also mitochondria containing coarse electron dense deposits in their matrix, probably iron (Figs. 3c, 4a). Moreover, there is often an extensive Golgi apparatus (Fig. 4b) surrounded by vesicles and tubules with a coating on the inner surface, possibly due to enhanced ferritin transportation. Coarsely granulated siderosomes and mitochondria with dense inclusions are also seen nearby (Fig. 4a). Most conspicuous are the abnormal clefts of the peripheral chromatin of the nuclei of erythroblasts which may be encountered in en-face aspects (Fig. 4c) or forming membrane bound splits (Fig. 4d).

Granulopoiesis

Light microscopy shows prominent proliferation with a shift to the left, an increase in mitotic figures and an extension towards the centers of the bone marrow space (Fig. 5a–c) in the 23 cases with the hypercellular marrow. The patients with hypocellular marrow, however, display a severe hypoplastic granulopoiesis with a delay in maturation. The neutrophilic granulocytes show dissociation of maturation as demonstrated by the frequent occurrence of a pseudo-Pelger-Huët anomaly (Fig. 6a). This abnormal hyposegmentation of the nucleus is often accompanied by an increase in eosinophilic granulocytes, including many precursor forms (Fig. 6b compare with Fig. 2a). Sometimes there is re-

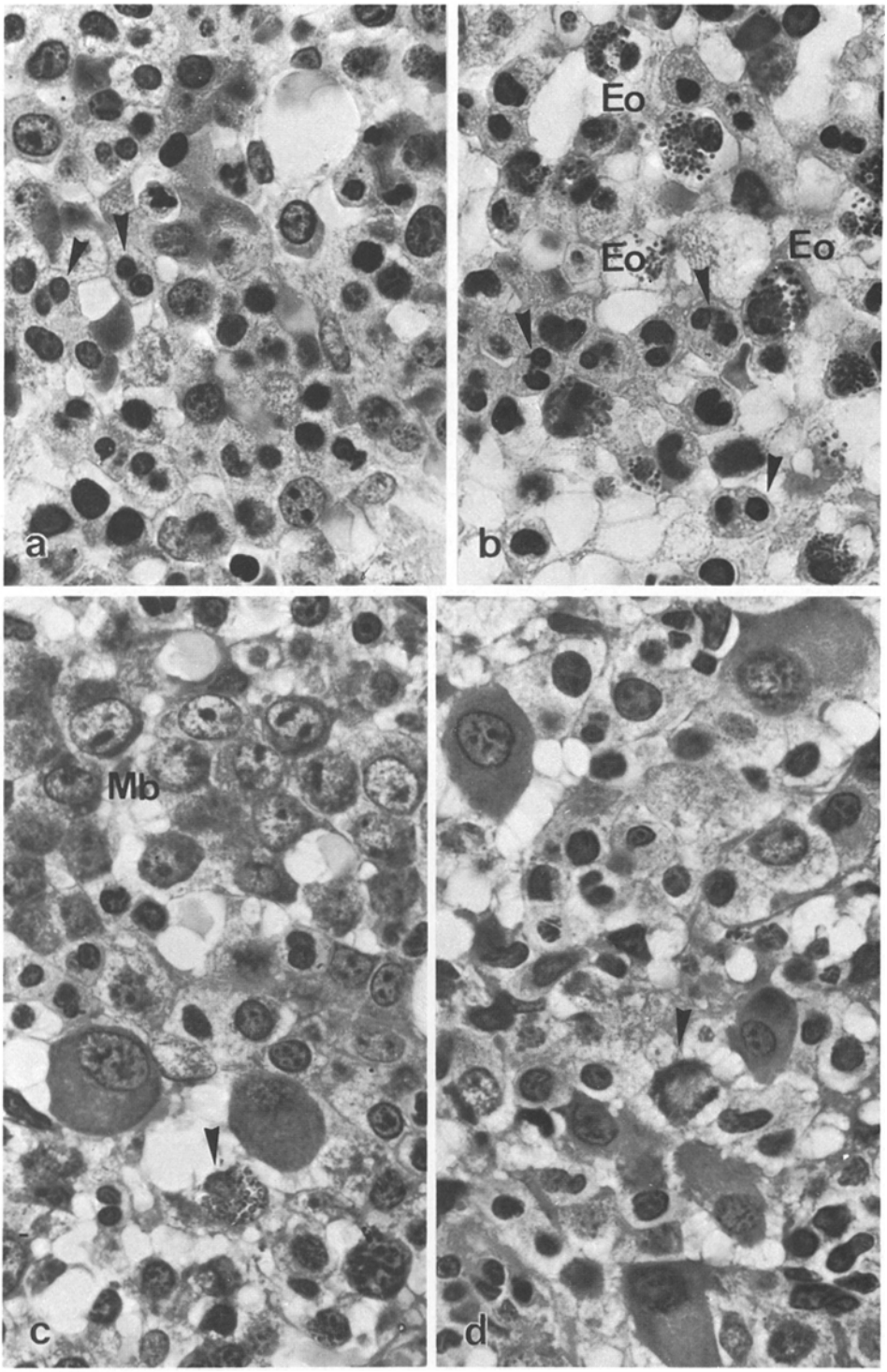


Fig. 6a–d. Light microscopy of neutrophilic and megakaryocytic granulopoiesis. **a** Neutrophilic granulopoiesis with several myelocytes and promyelocytes (*shift to the left*) and pseudo-Pelger-Huët anomaly of maturation (*arrow heads*). **b** Several eosinophilic myelocytes and metamyelocytes (*Eo*) and pseudo-Pelger-Huët anomaly (*arrow heads*). **c** Large clusters of megaloblasts (*Mb*) and two micromegakaryocytes and an eosinophilic metamyelocyte (*arrow head*). **d** Atypical micromegakaryocytes surrounding a mitotic figure (*arrow head*) and showing a lack of nuclear lobulation but a large proportion of cytoplasm (*upper half*). **a–d** ×980

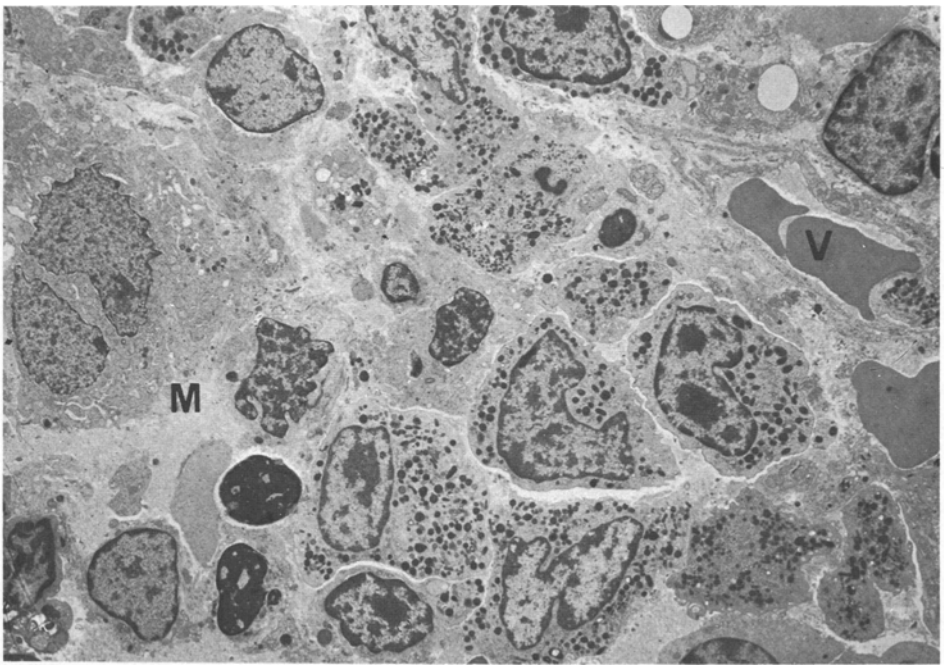


Fig. 7. Ultrastructure of granulopoiesis in myeloid dysplasia. Survey with many neutrophilic meta-myelo- and myelocytes with different amounts of granules and cytoplasmic/nuclear differentiation. Bilobated micromegakaryocyte (*M*) and vessel (*V*) filled with erythrocytes. × 5,500

markable monocytoid differentation of apparently neutrophilic granulocytes apart from frequently encountered mature monocytes.

Megakaryopoiesis is very prominent and is increased in the hypo- and hypercellular marrow specimens and characterized by atypical and even bizarre cells (Fig. 5b). These display either polymorphic microforms with a lack of nuclear lobulation (Fig. 6c) or small nuclei surrounded by a large area of cytoplasm and many mitotic figures (Fig. 6d) indicating nuclear-cytoplasmic dissociation of maturation. Besides their frequent arrangement along the sinuses there is often an abnormal dislocation into the peritrabecular generation zone of neutrophilic granulopoiesis.

Electron microscopy demonstrates neutrophilic granulocytes with remarkable differences in the number of granules in their cytoplasm and abnormal micromegakaryocytes (Fig. 7). Granulogenesis is irregular displaying, almost exclusively, granules of the secondary type in a lobated metamyelocyte with bizarre nuclear loops (Fig. 8a). Elsewhere large assemblies of round primary granules occur simultaneously with some elliptical secondary granules in a late myelocyte (Fig. 8b). The frequent eosinophils show extensive clusters of specific granules with a conspicuous crystalline core (Figs. 8c, d compare with Fig. 2a). As in the erythrocytic series the atypias of the nuclei are the most striking. These exhibit abnormal veil-like loops with inclusions of large areas of cytoplasm (Fig. 9a), small nuclear clefts (Fig. 9b) or very prominent nuclear appendages

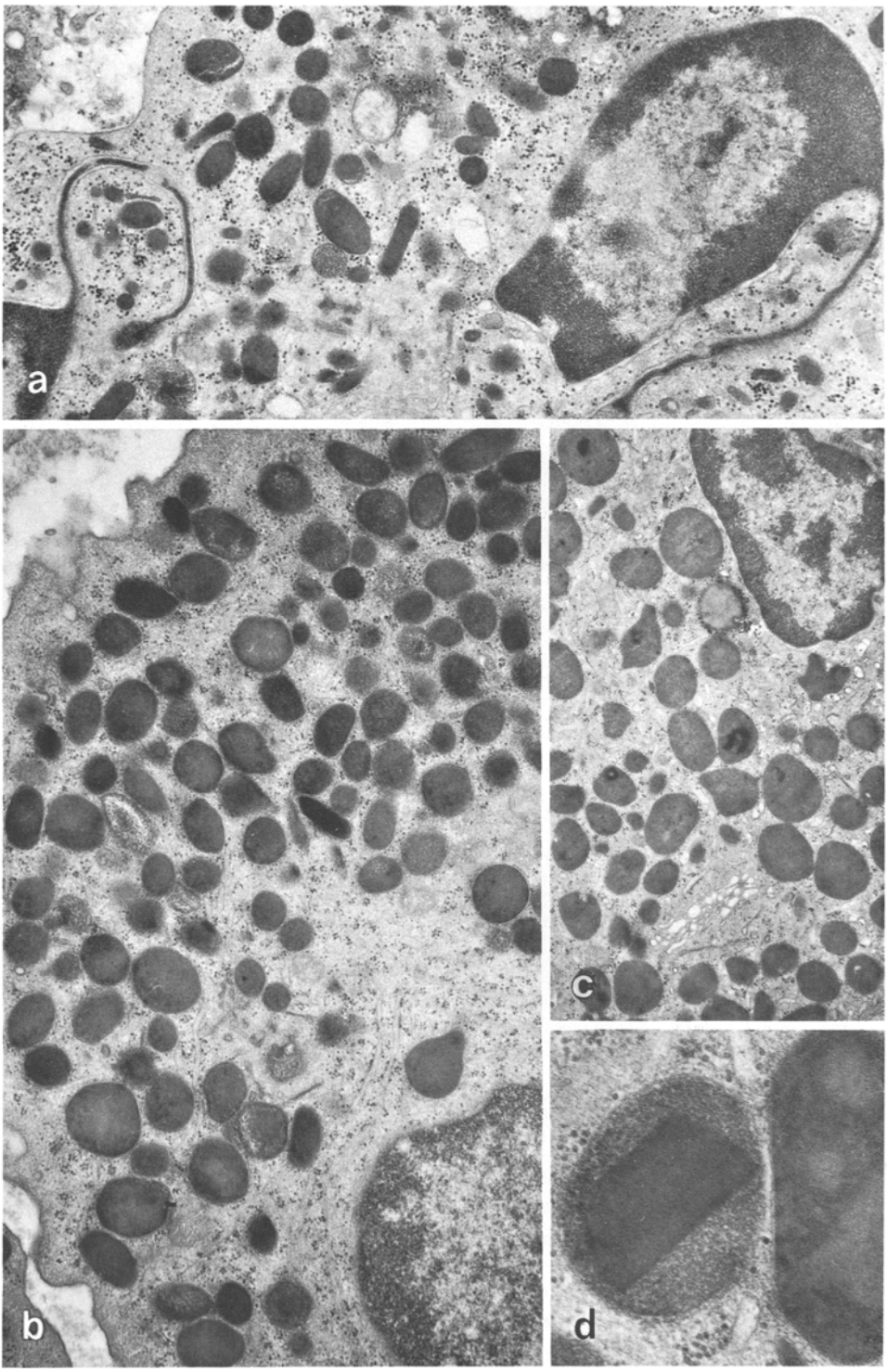


Fig. 8 a–d. Electron microscopy of granulogenesis. **a** Lobated metamyelocyte with abnormal nuclear loops and large granules mostly of the secondary type surrounding a Golgi area. **b** Huge amount of primary granules and some secondary in a late myelocyte. **c** Eosinophilic metamyelocyte with large specific granules showing an internal core or condensation. **d** Eosinophilic granule with central crystalline core. **a** and **b** $\times 18{,}000$; **c** $\times 15{,}000$; **d** $\times 45{,}000$

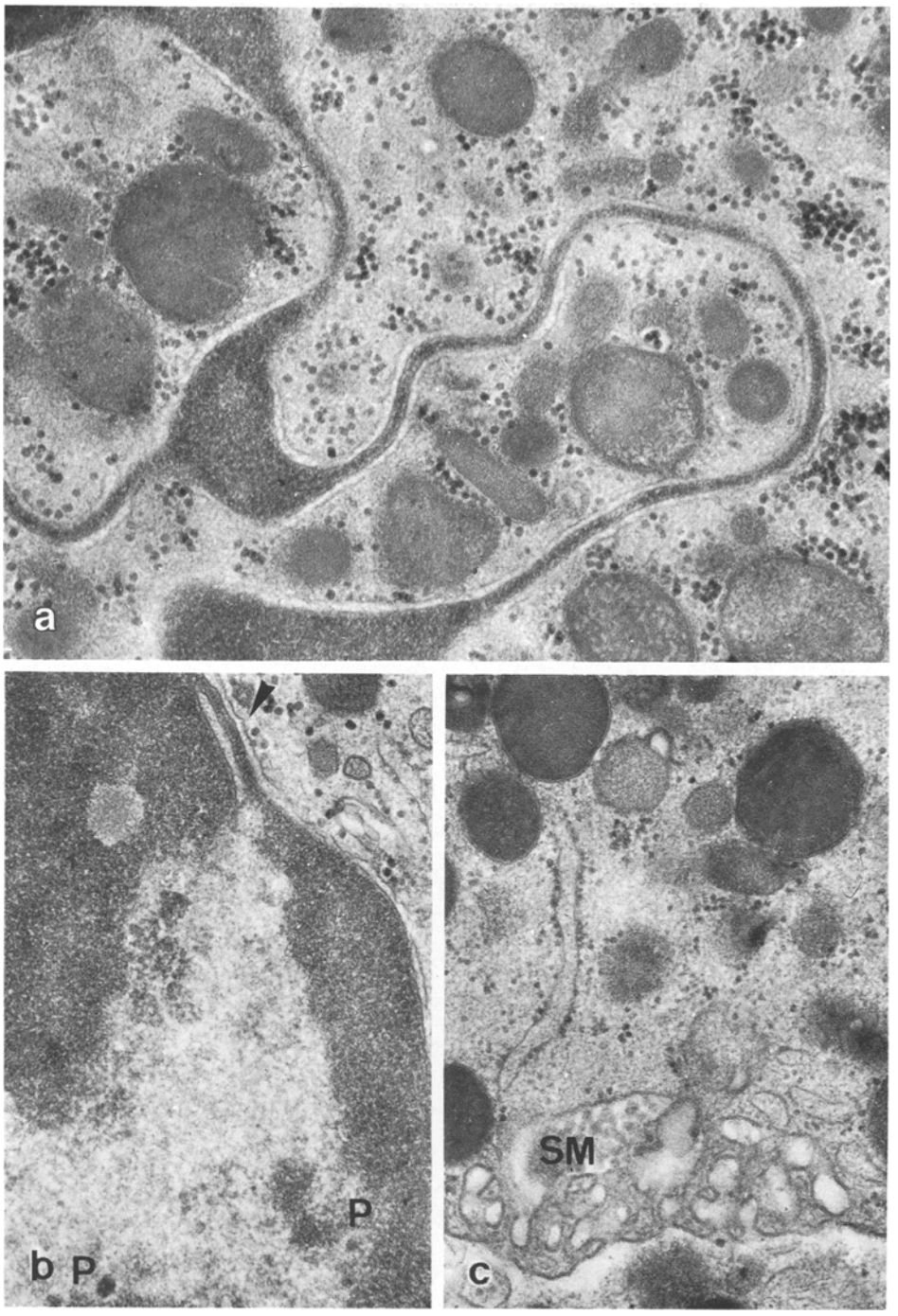


Fig. 9a–c. Atypias of neutrophilic granulocyte differentiation. **a** Large veil-like loop of a nucleus of a metamyelocyte apparently enclosing cytoplasmic regions. In the cytoplasm large round primary as well as more elongated secondary granules. **b** Small nuclear cleft (*arrow head*) in the heterochromatin of a metamyelocyte nucleus showing also large perichromatin granules (*P*). **c** Smooth membrane complex (*SM*) of a metamyelocyte forming a sponge-like structure possibly by invagination and subsequent branchings of the plasma membrane. **a–c** $\times 45{,}000$

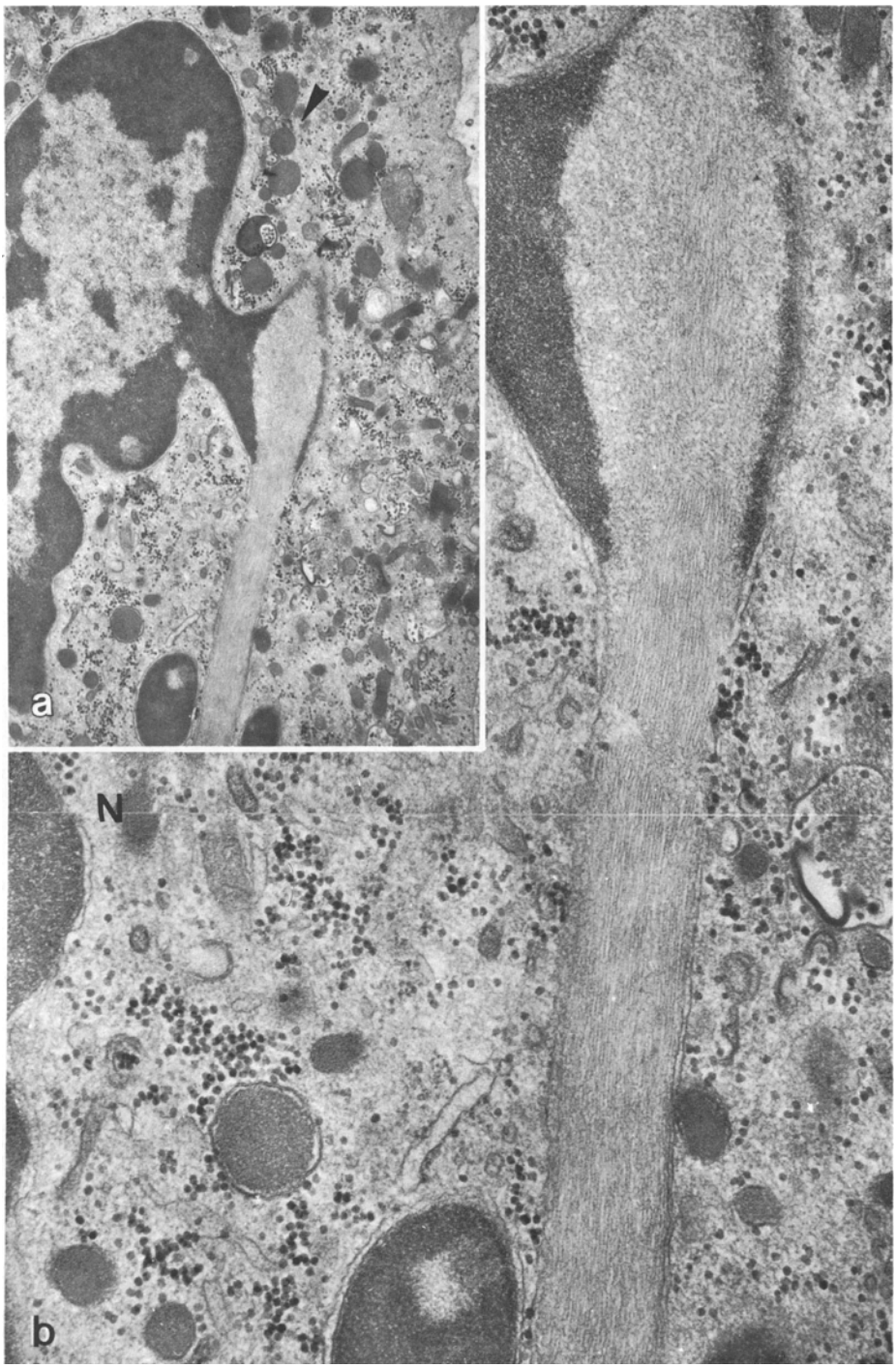


Fig. 10a and b. Atypias of neutrophilic metamyelocyte. **a** Many secondary and tertiary (dumbell shaped) granules in addition to a very few primary ones (*arrow head*) and a conspicuous nuclear appendage are seen. **b** This nuclear appendage shows a fibrillar interior and may be compared with a nuclear outrigger (or Nebenkern), peripheral segment of the nucleus (*N*). **a** ×12,000; **b** ×45,000

(so called Nebenkerne, Fig. 10a) with a regularly arranged inner fibrillar structure (Fig. 10b). Infrequently, in promyelocytes, the periphery of the cytoplasm has smooth membrane complexes (Fig. 9c). These remarkable infoldings resemble some kind of early demarcation membrane systems almost comparable with a megakaryocytic differentiation.

The atypical microforms of megakaryocytes display an ultrastructure which is compatible with a dissociation of maturation of the nuclei and particularly the demarcation membrane system causing a shedding of large and abnormal thrombocytes.

The *myeloid stroma* has a spongy appearance caused by edema which is patchy and frequently accompanied by a perivascular and conspicuous deployment of plasma cells along the presinusoidal capillaries (Fig. 5c). Sometimes there is also an increased necrobiosis of single cells or of small groups of neutrophilic granulocytes, with subsequent phagocytosis by histiocytic reticulum cells.

Following the first biopsy of the bone marrow at the time of diagnosis of the presumptive preleukemic state, the subsequent corings (Table 3) revealed an overwhelming proliferation of neutrophilic granulopoiesis and/or megakaryocytes, often with severe anarchy of maturation. This finally lead to CML in 10 patients (or its subgroups CGL and CMGM, see Georgii, 1979). In the 6 patients with a blast crisis of CML, the demonstration of a Philadelphia (Ph')-chromosome was possible in 5 cases (see Table 3). The last biopsy or the autopsy (Table 5) showed prominent blastic transformation of the bone marrow with massive infiltrates of liver and spleen. The sequential biopsies of the 11 cases with the final diagnosis of ANLL (Table 4) were characterized by an occasionally rapidly occurring transformation of the bone marrow by a diffuse growth of blasts. In the 3 autopsied patients (Table 5) there was a remarkable systemic organ manifestation with massive infiltrates of liver, spleen and lymph nodes by blasts and a blastic transformation of the bone marrow at the various sites investigated. The causes of death were pneumonia, sepsis and cerebral hemorrhage due to insufficiency of the bone marrow. Finally it should be noted that hypo- or hypercellular marrow was directly transformed into the leukemic condition and that our sequential trephine biopsies gave no evidence for a change in cellularity before the onset of leukemia.

Cytogenetic studies demonstrated a Ph'-chromosome in 4 cases of CML prior to overt leukemia and Ph' negative cases (Tables 3 and 4, see also Thiele et al. 1979a). It was remarkable that the presence of the Ph'-chromosome preceded the onset of CML between 4–16 months, as judged by clinical findings and histopathology of the bone marrow.

Discussion

The *histopathology* of cores of bone marrow in the preleukemic state which is called myeloid dysplasia (MD) preferentially, is only partially compatible with the results of marrow aspirates. The findings demonstrated in cases of preleukemia as precursors of ANLL by several authors (Saarni and Linman 1973; Fisher et al. 1973; Linman and Saarni 1974; Linman and Bagby 1976,

1978; Majolino et al. 1978) were also described in a preliminary report (Thiele et al. 1979a) and have been observed by Fischer and Schaefer (1979) in the bone marrow of patients with preleukemic syndromes preceding AML. Precise evaluation of the three cell lineages of hematopoiesis and the myeloid stroma can be obtained by semithin sections of plastic embedded core biopsies, especially in repeated examinations. Thus thorough evaluation of all those changes in cytomorphology which are not completely revealed in smears of bone marrow aspirates is feasible. Further abnormalities of hematopoiesis may be detected by the examination of these core specimens and a comprehensive description of all cellular elements of the bone marrow including alterations of the microtopography is possible. From our experience of these 27 patients, it may be concluded that the prephases of ANLL and CML and their blastic transformation, are identical. The fact that preleukemia (myeloid dysplasia) can be observed as a precursor lesion of CML has been reported in a previous observation. Heimpel et al. (1979) mentioned one patient in a series of 33 cases with preleukemia, who in the course of 5 years developed CML and later evolved a blast crisis comparable with several of our cases (Table 1 and 3).

The megaloblastoid *erythropoiesis* and sideroblasts are particularly prominent features (Saarni and Linman 1973) confirmed by electron microscopy. The abnormal iron deposits in the mitochondria of the erythroblasts have been described by Maldonado et al. (1976) in cases of refractory anemia and preleukemia. The coarse clumps of ferritin are consistent with the intermediate forms of sideroblasts (Bottomley 1977) which are often encountered in aregenerative anemia with a hypercellular marrow, leading to acute leukemia in a high percentage of patients (Hast 1978). In addition to these disturbances of iron metabolism the ultrastructure of nuclear atypicality is another, more striking indication of a maturation defect of erythropoiesis, as similar alterations have been recorded in non-treated cases of polycythemia vera (Thiele et al. 1979b), so called refractory anemia (Maldonado et al. 1976) and dyserythropoiesis in aplastic anemia (Frisch et al. 1975). Moreover, the obvious disarrangement of normal cellular development and metabolism is shown by the findings of atypical erythroblast and iron kinetics in human preleukemia (Queisser et al. 1972; Hast and Reizenstein 1977) and colony formation in vitro (Koeffler et al. 1978).

Neutrophilic *granulopoiesis* is often characterized by an acquired pseudo-Pelger-Huët anomaly of granulocytes, a common and well known feature of acute and chronic myeloproliferative diseases (Dorr and Moloney, 1959). The defective enzyme equipment of apparently mature cells shown by decreased activity of peroxidase, alkaline phosphatase or naphtol-AS-D-chloroacetate-esterase (review by Schmalzl et al. 1978) may be related to the abnormal granulogenesis of the neutrophilic lineage. This suggestion is further supported by the demonstration of cytochemical and ultrastructural aberrations of neutrophilic granules (so called paraneutrophilic granulocytes, Fischer and Schaefer 1979). In addition Breton-Gorius et al. (1976) reported similar abnormalities of nuclear segmentation, bleb formation and especially smooth membrane complexes as found in our patients in two cases of preleukemia. The peculiar fibrillar appendages of the nuclei and the other structural atypias are consistent with the electron microscopical findings frequently detected in granulocytes of acute

and chronic myeloid leukemia (Bessis and Breton-Gorius, 1969). Thus the appearance of abnormal micromegakaryocytes as marker cells of impending leukemia is only in context of this anarchy of hematopoisis. Similar microforms of megakaryocytes and large or giant platelets have been encountered in preleukemia and myelomonocytic leukemia by Smith et al. (1973), Maldonado (1975, 1976) and Thiele et al. (1977) in chronic megakaryocytic-granulocytic leukemia (CMGM).

All these morphological features observed in erythro- and granulopoiesis in preleukemia corroborate experimental findings (cell cultures, ^{3}H-thymidine labelling, cytophotometry), suggesting a maturation defect. This defect is manifested functionally as ineffective hematopoiesis during that period when the neoplastic clone is established (Golde and Cline 1973; Koeffler and Golde 1978). The abnormalities of cellular development are seen in megakaryocytes (Queisser et al. 1972, 1974), erythrocytes (Queisser et al. 1972) and neutrophilic granulopoiesis (Senn and Pinkerton 1972; Senn et al. 1976). This concept of a maturation defect with morphological expression in all three cell lineages before transformation into histologically and clinically apparent leukemia is supported by the finding of a Ph'-chromosome and aneuploidy in some of these cases (Thiele et al. 1979a).

The period of a preleukemic state may be very short, particularly in those patients who develop ANLL. In our cases it ranged from 2 to 30 months while others reported 2 months (Fisher et al. 1973; Fischer and Schaefer 1979), less than 4 months (Schmalzl et al. 1978) or 6 months (Saarni and Linman 1973; Linman and Bagby 1978). Most remarkable however, is the significantly longer period of preleukemia in patients who develop CML (see Table 6); an insidious onset may be one of the main reasons why a preleukemic stage is rarely recognized preceding CML.

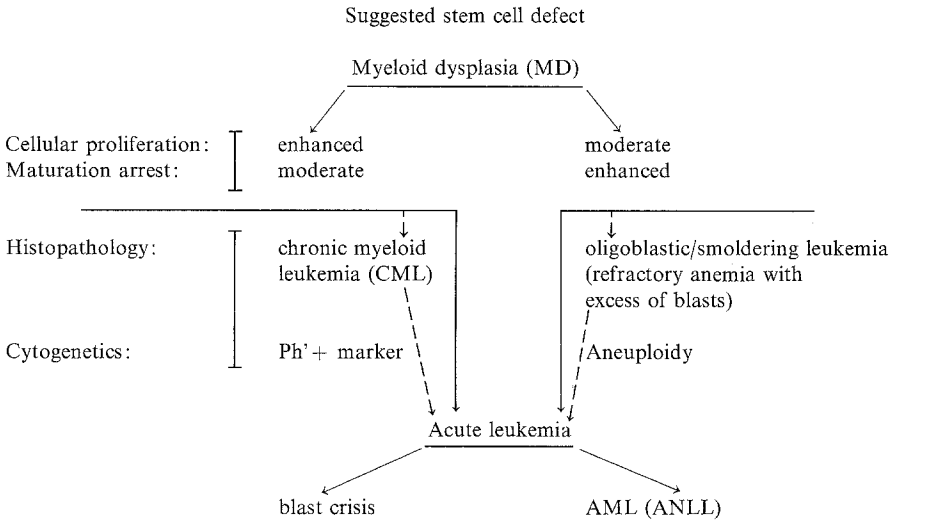
The morphological features of myeloid dysplasia in the bone marrow which represent potential precursor lesions of acute or chronic myeloid leukemia should be separated from entities which are sometimes regarded as preleukemic states: smoldering acute leukemia (Rheingold et al. 1963), oligoblastic leukemia (Bernard et al. 1975; Izrael et al. 1975) and so called refractory anemia with an excess of myeloblasts in the bone marrow (Dreyfus 1976; Najean and Pecking 1977). The common feature of all these disorders is an increased number of myeloblasts (at least 10% and more) observed in smears of bone marrow. Those 3 entities should not be confused with myeloid dysplasia, since there is no significant increase of myeloblasts in that lesion. For this reason synonymous use of myeloid dysplasia for preleukemia, smoldering acute leukemia, oligoblastic leukemia and refractory anemia (with an increase of blasts) should be avoided. These 3 alterations are early stages of a clinically concealed leukemia.

In our 195 cases of clinically suspected preleukemias at least 5 cases presented with an increased number of blasts in the bone marrow along the peritrabecular generation zones of granulopoiesis at the time of the first biopsy. In the follow up studies these cases evolved into AML (ANLL) proven by clinical data and the sequential examinations of the bone marrow. They were thus regarded as belonging to those entities of early stage or oligoblastic leukemia and excluded from our study. These cases confirm the results of Heimpel et al. (1979) on

Table 8. Differential diagnosis of some disorders of the bone marrow as derived from core biopsies, which may be erroneously confused with myeloid dysplasia

Histology / Disorder	Cellularity	Erythropoiesis	Granulopoiesis	Megakaryopoiesis	Iron deposits	Myeloid stroma
Aplastic syndrome (aplastic anemia, panmyelophthisis, hypoplastic panmyelopathy)	↓↓ diffuse	↓ delay of maturation, shift to the left, megaloblastoid	↓ toxic granulation, vacuoles of nuclei and cytoplasm, necrobiosis +	↓↓ overaged (pyknotic)	sideroblasts +/−	lymph follicles, frequent mast cells and plasma cells, edema of interstitium
Hyperplastic panmyelopathy	↑↑ diffuse	↑↑ arrest of maturation, shift to the left, megaloblastoid	↓shift to the left	↓ overaged (naked nuclei, pyknotic forms)	sideroblasts +/−	perisinusoidal plasmacytosis
Megaloblastic anemia (pernicious anemia)	↑↑ diffuse	↑↑ megaloblasts, nuclear fragmentation (Howell-Jolly bodies)	giant forms, hypersegmentation vacuoles of cytoplasm	↓ hyperlobulation of nuclei	reticulum cells +	−
Sideroachrestic anemia (sideroblastic anemia)	↑↑ diffuse	↑ arrest of maturation, megaloblastoid	normal	normal	ring sideroblasts +++	
Autoimmune disease (hyperergic or hypersensitivic myelopathy, LEV)	↓ or normal diffuse	↓ or normal, delay of maturation	↑ or normal eosinophils, necrobiosis ++ nucleophagocytosis (LE-phenomenon)	normal, overaged forms	reticulum cells +++	prominent perivascular plasmacytosis and exudative panvasculitis, frequent mast cells, edema of interstitium
Oligoblastic leukemia (smoldering leukemia)	↓ diffuse	↓ shift to the left, severe arrest of maturation, megaloblastoid	normal or ↑ arrest of maturation (hiatus), increase of blasts and atypical monocytoid forms	frequent polymorphism atypical microforms	sideroblasts +	edema of interstitium
Myeloid dysplasia (MD)	↑ infrequently ↓	↑ delay of maturation, macrocytic-megaloblastoid	localized hyperplasia, shift to the left, arrest of maturation, frequent eosinophils, pseudo-Pelger forms	↑ polymorphous, atypical microforms	sideroblasts ++	perisinusoidal plasmacytosis, edema of interstitium

Table 9. Suggested position of myeloid dysplasia in the system of myeloproliferative disorders



the course and prognostic criteria of preleukemic stages depending on an increased myeloblast count. On the other hand, several inflammatory or hyperergic hematological disorders and even pernicious anemia may be erroneously mistaken for myeloid dysplasia. The differential diagnosis in the histopathology of these lesions is listed in Table 8.

Reviewing the fine structure of the bone marrow by light- and electron microscopy and the results of cytogenetic investigations, the clinically defined term preleukemia should be replaced by *myeloid dysplasia (MD)*. In comparison with and contrast to the term hemopoietic dysplasia (Bessis and Brecher 1976; Fischer and Schaefer 1979) this indicates a lesion which may possibly, and later, transform into *acute* and *chronic* myeloid leukemia. This concept of myeloid dysplasia in the system of myeloproliferative disorders is given in Table 9.

It should be emphasized that the definition of myeloid dysplasia is derived from the histopathology of the bone marrow and does not necessarily imply evolution of leukemia after a prolonged period of time. This entity occurs only in a high risk group of patients that should be followed clinically by a close out-patient service. A further argument for avoiding the term preleukemia is its limitation to the later development of ANLL, according to the commonly understood definition. This limitation to acute leukemia should be re-evaluated, since it is well known that the terminal phase of CML, the blastic crisis may present clinically as ANLL (Bloomfield et al. 1977). A lymphoblastic transformation may also occur in CML (review by Janossy et al. 1979) or even a megakaryoblastic crisis (Breton-Gorius et al. 1978). Finally the differences in the various disorders of the bone marrow which are frequently covered by the clinicians by the term preleukemia, which actually range from oligoblastic leukemia to sideroblastic anemia (see also Table 8) should be replaced by a more clear cut definition, depending on histopathology of bone marrow biopsies.

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