Histopathology of Bone Marrow in Human Chronic Leukemias

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Chronic myelogenous leukemias have arisen a considerable new interest, since lymphoblastic crises recently turned out to complicate and terminate a primary myeloproliferative disease (Beard et al., 1976; Janossy et al., 1977; Rosenthal et al., 1977). Ways of stem cell differentiation (Metcalf, 1973, 1974, 1977; Boggs, 1974; Moore, 1976; Greenberg, 1976, 1978) as well as biological markers of leukemic cells (Fialkow et al., 1977, 1978a, b) give sufficient proof for the suggestion that hematopoietic stem cells have been primarily involved in this disease.

The morphology of leukemias is based on histopathology, cytochemistry and electron microscopy, whereas gross pathology is of lesser importance for their determination. There is a large variety of subgroups from chronic leukemia – CML – which may be classified comprehensivly only by the histopathology of biopsies from bone marrow cores. Thus it is not clearly known which pathogenetic relationships are existing between the numerous subgroups, as erythro- and megakaryocytic leukemias for instance, and how they are related to each other and to CML. Moreover the pathways are not sufficiently understood by which CML and its subgroups or other myeloproliferative diseases, as Polycythemia vera, terminate into final stages of myelofibrosis and myeloid metaplasia (Gralnick et al., 1971; Ward and Block, 1971; Buyssens and Bourgeois, 1977).

A more detailed morphology of chronic myelogenous diseases ought to bring up new insights to these basic questions, provided histopathology is supported by electron microscopy (Thiele et al., 1977a, b, c), chromosome analysis (Sandberg and Hossfeld, 1970; Rowley, 1976, 1978) and completed with results of enzyme markers (McCaffrey et al., 1975; Gallo, 1975) and membrane phenotypes (Greaves, 1975) and compared with clinical findings.

The acute leukemias, however, can be characterized according to their cellular composition by methods of membrane (Janossy et al., 1977; Catovsky and Galton, 1977; Catovsky et al., 1978; Gordon and Hubbard, 1978) and enzyme markers (Hoffbrand et al., 1977; Mertelsmann et al., 1978) alone with more reliability than morphological methods are able to do. Even the enzyme cytochemistry (Leder, 1975; Bennett and Reed, 1975; Bennett et al., 1976; Löffler, 1978; Schmalzl et al., 1977) electron microscopy (Bessis, 1973, , 1975) and chromosome analysis (Golomb et al., 1976; Alimena et al., 1977) are only of a supporting value to characterize acute leukemias but do not offer a final diagnostic clue.

For these reasons this study will be restricted to the histopathology of biopsies from bone marrow cores in chronic myelogenous leukemias and other myeloproliferative diseases and is supported by findings from chromosome analysis and by former results of electron microscopy as well as clinical findings in these same patients.

Material and Methods

Results of this study are based on core biopsies of bone marrow from the anterior iliac crest by the method of Burkhardt (1966a, b) and Georgii and Thiele (1976) of the posterior iliac crest by the method of Jamshidi and Swaim (1971). Resin embedding was done using methacrylate, semi-thin sections of 3 μ were stained with the usual procedures of hematopathology as elsewhere reported (Georgii and Thiele, 1976; Vykoupil et al., 1976). Electron microscopy by thin section and freeze-fracture techniques was formerly described (Georgii and Thiele, 1976) as have been methods of chromosome analysis in short term cultures from bone marrow cells which were obtained from the puncture sites after withdrawing the core of the biopsy (Krmpotic et al., 1968; Golomb et al., 1976). All biopsies were done before any therapy and selected from routine samplings out of a pool of 7,000 patients, among which, 718 cases with chronic leukemias or myeloproliferative diseases and 190 with acute leukemias were found.

Results

The own material shows a prevalence of chronic to acute diseases in a relation of 3 : 1, which is another reason to restrict this study to chronic myeloproliferative disorders. These disorders can be classified into 9 subtypes including the Polycythemia vera and unclassifiable entities (Table 1).

Table 1. Distribution of chronic myeloproliferative disorders in a total of 718 patients found in 7,000 sequential biopsies of bone marrow: There is a conspicuous group of myeloses with mixed cellularity, so called chronic megakaryocytic granulocytic myelosis – CMGM

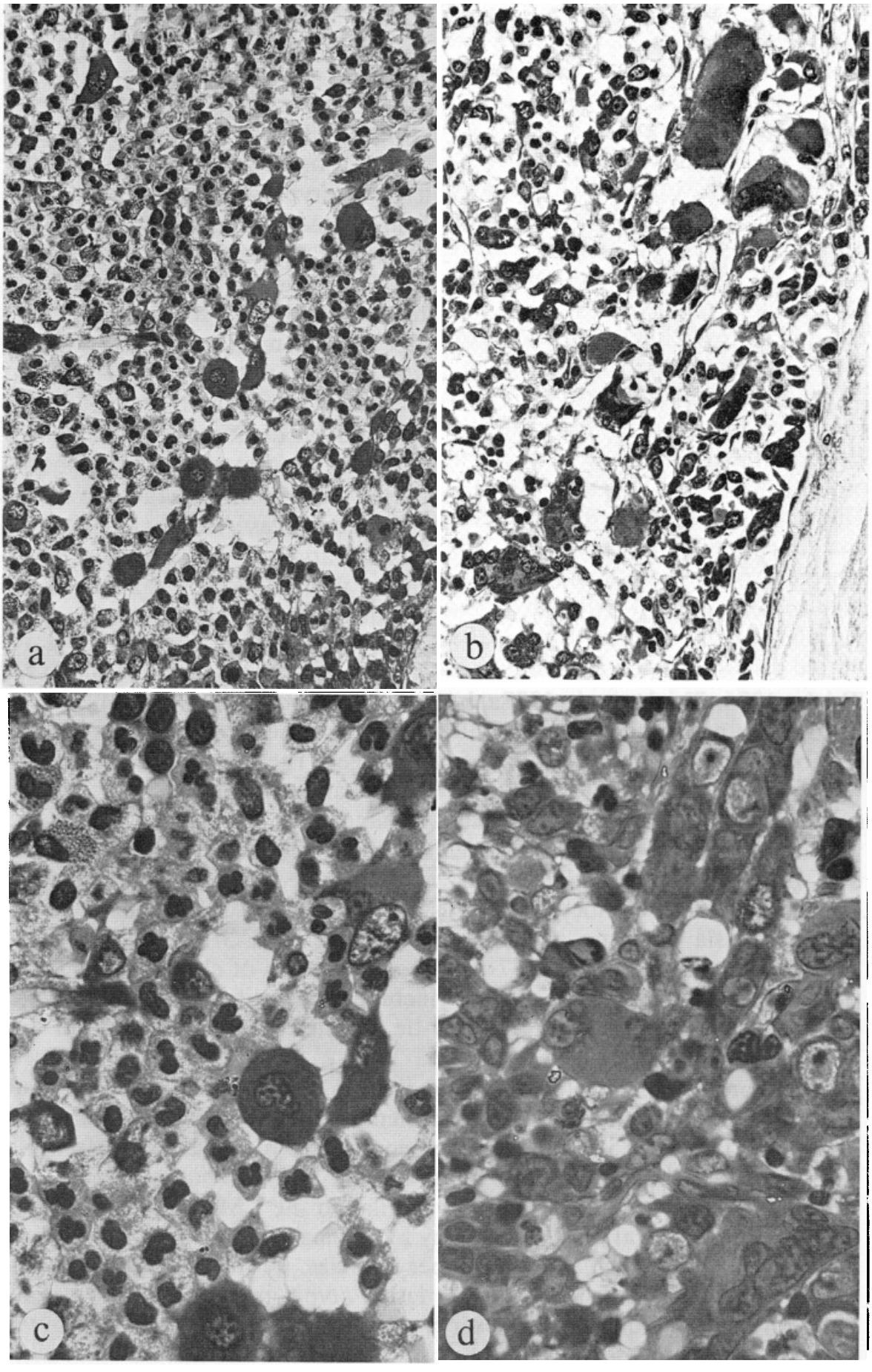
Chronic leukemias		n	%
Chronic granulocytic leukemia – CGL –		133	19
Smouldering leukemia		5	1
Mixed myeloses	– CMGM Ia. II –	187	26
Myelofibroses	– CMGM IIIa. IV –	238	33
Primary myelofibroses		14	2
Thrombocythemia		11	2
Myelo-monocytic leukemia		3	0
Polycythemia vera		65	9
Unclassified		62	9
Total		718	100

Among chronic myelogenous leukemias – CML – the chronic granulocytic leukemias – CGL – are those with a neoplastic proliferation of granulopoiesis. This atypical granulopoiesis is gradually replacing the fat tissue of the bone marrow space (Fig. 1a). The erythro- and megakaryopoiesis are not seriously reduced, but left in a sufficient amount, which is in contrast to the acute leukemias of ALL, AML or ANLL-type. The cellular differentiations of granulopoiesis is fairly preserved whereas precursors as promyelocytes and myelocytes are increased. Myeloblasts are not proliferating in remarkable amounts and their increase points towards a beginning transformation into a blastic crisis. Following this description the CGL may be defined as a neoplastic growth of only one cell lineage, while the other 2 or 3 lines remain at least superficially unchanged or may turn into a reactive hyperplasia of megakaryocytes in rare cases. This subgroup of typical CGL's amounts to 19% in our series of 718 patients (Table 1). From the CGL the group of myeloses should be distinguished which are characterized by an additional neoplastic proliferation of the megakaryopoiesis, thus resulting in a mixed cellularity from 2 cell lineages involved. The histology shows a remarkable numerical increase of megakaryocytes and their precursors (Fig. 1b). These cells are atypical with enlarged pleomorphic, non-pyknotic nuclei which differ from typical megakaryocytes even in CGL (Figs. 1c, d). The immature nuclei in a well developed mature cytoplasm cause a dissociation of the differentiation as formerly demonstrated in ultrastructural studies of bone marrow biopsies (Georgii and Thiele, 1976; Thiele et al., 1977a, b). The granulopoiesis is altered likewise, but mostly not to the same extend as in typical CGL. The erythropoiesis can be increased in some cases of early stages. The marrow mesenchyme with sinuses and reticulin fibers remains unchanged during the early stages of development, which was designated as stage I (Fig. 1b).

Based on the neoplasia of two cell lineages which do proliferate in a slow and fairly well differentiated way, we have called this entity a "chronic megakaryocytic granulocytic myelosis" – CMGM – in contrast to the one-line neoplasia "chronic granulocytic leukemia" – CGL – (Georgii and Thiele, 1976; Thiele et al., 1977b, c). The usual term of chronic myelogenous leukemia – CML – may probably include both entities the CGL and the CMGM too; since the latter can only be detected by a core biopsy of the bone marrow.

There are 2 other myeloproliferative diseases that should be strictly distinguished from this entity CMGM: Polycythemia vera and Idiopathic Thrombocythemia. The latter is a one cell line neoplasia of megakaryopoiesis while the other lines show an inconspicuous growth. The histopathology of bone marrow in Polycythemia vera is very similar to the CMGM, in spite of its very different clinical findings. Only by hyperplasia of erythropoiesis and of sinuses, which are increased in number and size, by the complete absence of stainable iron in histocytes and a lesser atypia in granulo- and megakaryopoiesis the experienced observer may reach the diagnosis of Polycythemia vera in non-treated cases.

The pathogenesis of CMGM may be observed by repeated biopsies dur-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ing the course of this disease. An increase of fine reticulin fibers in a discrete patchy pattern may be found by investigating silver stained slides in the polarizing light microscope. With an increasing number of reticulin fibers the disease is classified as stage II of CMGM; this shows a slow progress which may take many months, sometimes exceeding one year and more (Fig. 2a). This stage continues to proceed into a diffuse reticulin lattice which is extended all over the marrow space, polymerizing into collagen bundles which represent the onset of fibrosclerosis; this is defined as stage III of CMGM. The stage IV finally includes an additional endophytic growth of bone trabeculi while the reticulin changes into complete fibrosclerosis. Even in these final stages the neoplastic proliferation of megakaryocytes and granulocytes can be seen, which is, in regard to the proliferating cells, still similar to stages I and II (Fig. 2d).

Lymphonoduli aggregati consisting of loose assemblies of small lymphocytes, mostly localized in the centers of the marrow space and not at paratrabecular sites, can be found frequently in these CMGM's of the first 3 stages (Fig. 2a). These lymphonoduli are rare in the final stage IV of CMGM and very rare in pure CML's.

Chromosome Analysis

In these studies the Ph^1-chromosome was found in 75% (15 from 20) of CGLs and in about 70% (15 from 23) CMGM's of the stage I and II (Table 2). The myelofibroses and osteomyelofibroses, which are classified as CMGM III and IV, do show the same frequency as CGL's, i.e. 75% (15 from 20). The technique of Giemsa-banding displays a 9:22 translocation as the most frequent aberration. Besides there are some minor anomalies as aneuploidy and breaks without clonal evolution. The primary idiopathic myelofibrosis and Polycythemia vera were found to lack a Ph^1-chromosome with one exception in Polycythemia vera (Table 2).

Clinical Findings

The CGL differs from CMGM in clinical finding especially of the cell counts from the peripheral blood (Table 3). The values of leukocytes in CGL's are

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Fig. 1a–d
a Chronic granulocytic leukemia – CGL – well differentiated granulopoiesis and normal looking but increased megakaryocytes. **b** Chronic megakaryocytic granulocytic myelosis – CMGM – megakaryocytes are very increased, display severe atypias and are dislocated from their usual sites to paratrabecular areas, closely to the terminal sinuses. **c** CGL, higher magnification from Fig. a, neutrophil granulocytes metamyelocytes and eosinophils can be seen. Megakaryocytes do not show striking alterations. **d** CMGM with severe polymorphism of megakaryocytes and precursors; they are enlarged in size and abnormal (compare with same magnification as Fig. 1c)
Magnifications: a, b ×350; c, d ×875. Staining: Giemsa

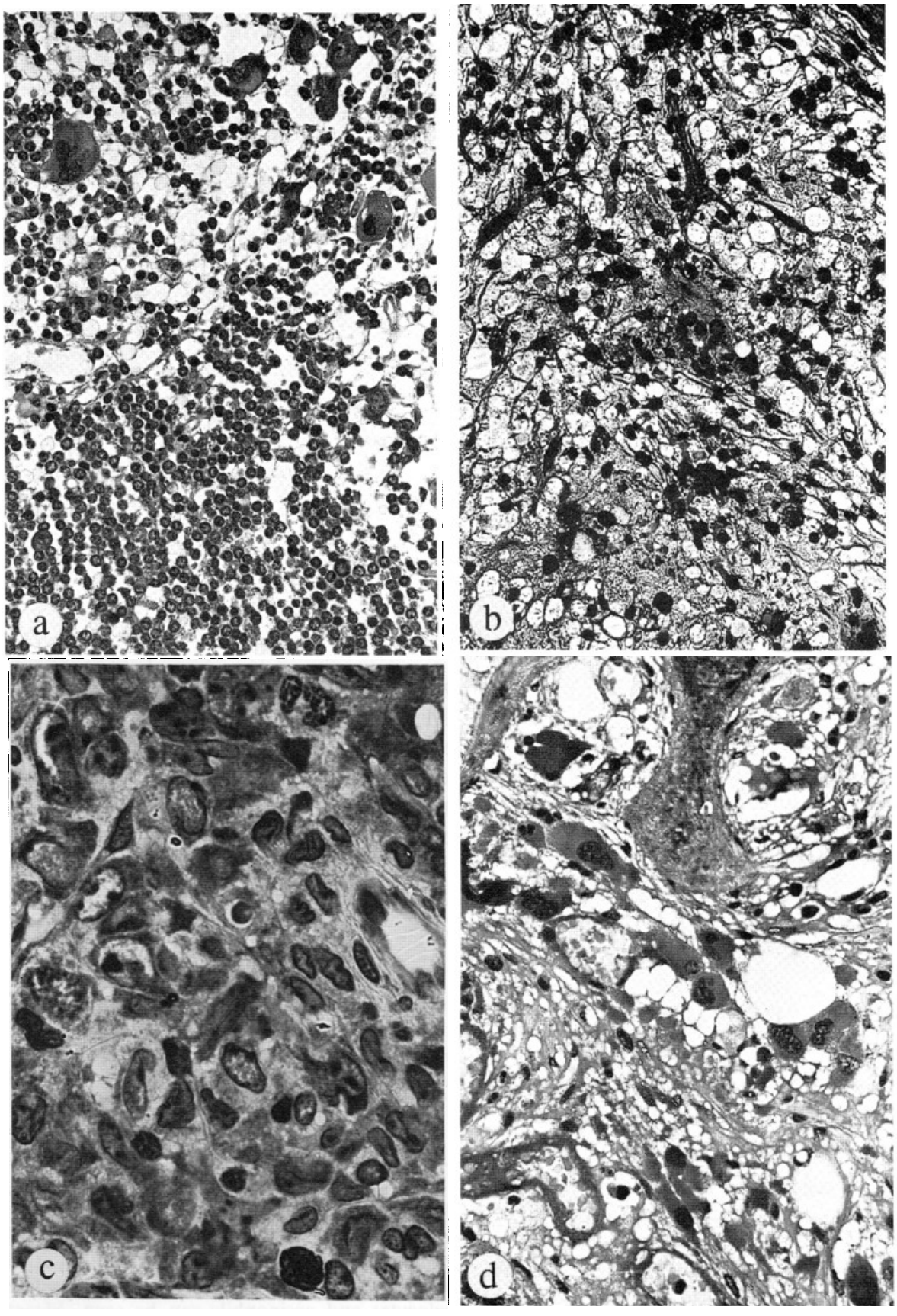
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Table 2. The Philadelphia-chromosome in chronic granulocytic leukemia (CGL) compared with chronic megakaryocytic granulocytic myelosis (CMGM), primary myelofibroses, and polycythemia vera in a total of 74 patients

Histology	Ph^1-positive	Structural anomalies	Aneuploidy	No changes
Chronic granulocytic leukemia	15/20	1/20	1/20	3/20
Mixed myeloses (i.g. CMGM Ia. II)	15/23	1/23	2/23	5/23
Polycythemia vera	1/10	0/10	8/10	1/10
Myelofibroses (i.g. CMGM IIIa. IV)	15/20	0/20	0/20	5/20
Primary myelofibroses	0/1	0/1	1/1	0/1

significantly higher than in CMGM of stage I and II, and even compared with stages III and IV which resemble myelofibrosis and osteomyelofibrosis. The thrombocytes seem to be conspicuously increased, but this is not significant statistically. The leukocytic alkaline phosphatase is elevated in CMGM's compared with CGL's, which seems to be important. – There is a significant difference of the mean age from CGL to CMGM patients but not among the CMGM's themselves. The prolonged prediagnostic time points to an insiduous onset and retarded natural course of CMGM's which must be ascertained by further clinical studies.

The difference of some clinical complications may be understood from the varying cell counts in the peripheral blood and by the divergent cellular growth shown by histopathology of the bone marrow. Bleedings to a severe and sometimes letal extent were observed in CGL's only – 6/24 – whereas they are missed in all CMGM's – 0/47 –. A thrombotic diathesis was found in CMGM's stage I and II only – 5/24 – while stages III and IV – 0/23 – as the pure CGL – 0/24 – were free of this complication.

Blastic crisis of CMGM (see Fig. 2c) can occur during all of their four stages but they are much more frequent in pure CGL's: in a mean of 13,7%, i.e. = 41/298 patients – of all stages from CMGM compared to 48% – 55/115 – in CGL patients blastic crises were observed.

Fig. 2a–d
a CMGM, this is stage II with slight, scattered increase of reticulin fibers, which can be detected even in Giemsa staining by the widening of sinuses. Noticeable are the lymphocytes forming a nodular infiltrate (lower half of the illustration). **b** Idiopathic, primary myelofibrosis with hyperplastic erythropoiesis with maturing arrest, but no hyper – or neoplasia of granulo – or megakaryopoiesis. This should not be termed a myeloproliferative disorder. **c** Blastic crisis of CMGM with precursors of megakaryocytes and to a lesser amount of granulopoiesis. **d** CMGM stage IV, usually called osteomyelofibrosis with newly formed bone trabecula, fibrosclerosis, widened sinuses, atypical megakaryocytes, there are rests of erythropoiesis and almost no granulopoiesis is shown in this area.
Magnifications: a, b, d ×350; c ×875. Staining: Giemsa

Table 3. Values of cell counts from peripheral blood compared with histopathology of bone marrow from CGL's and myeloses of mixed cellularity, chronic granulocytic megakaryocytic myelosis (CMGM); also age of patients and time between beginning of clinical symptoms and diagnostic biopsy are compared

	CGL	CMGM Stage I	CMGM Stage I a. II	CMGM IIIa. IV i.g. Myelofibrosis
Leukocytes				
mean	160	26.6	25.3	15.5
range	8.6–780	1.1–270	1.1–270	1.3–77
n	24	32	75	76
Thrombocytes				
mean	364.5	762	551	241
range	45–986	29–3000	8–3000	1.7–1321
n	24	30	64	68
Erythrocytes				
mean	4.26	4.32	4.16	3.28
range	2.2–5.3	1.4–6.5	1.4–7.1	1.6–5.7
n	24	29	70	63
Alkal. phosphat. in leukocytes				
mean	24.9	121	144	116
range	0–188	3–343	0–360	0–393
n	21	23	49	37
Age				
mean	53.1		63.4	60
range	15–84		26–85	25–84
n	79		87	91
Time – month				
mean	17.4		29.1	38.4
range	1–74		1–146	1–264
n	24		63	64

Discussion

The myeloproliferative disease described here as a chronic megakaryocytic granulocytic myelosis – CMGM – has been known for a long time under the term of a leukemic megakaryocytic myelosis or leukemia and was described mainly in case reports (Rappaport, 1966, cit. in Georgii and Vykoupil, 1972, 1976). The majority of these cases were only detected in acute blastic phases and therefore most frequently counted among acute leukemias as could be shown by Bain et al. (1977). Modern techniques of obtaining the biopsies and processing the bone marrow with semithin section have changed this opinion. The chronic diseases are very frequent (Table 1). We conclude from our personal experience that most acute looking cases are actually blastic crisis of chronic diseases. Real acute megakaryocytic myeloses are extremely rare, if they do exist anyway. Furthermore the semithin sections as well as electron microscopy have shown that granulopoiesis is also involved in this myeloproliferation.

The demonstration of Philadelphia chromosomes in metaphases from bone marrow cells implies a specific aberration associated with chronic myelogenous leukemia – CML – (Rowley, 1976). In about 90% of patients the Ph^1-chromosome should be found, which is the result of a 9:22 translocation in over 90%, as Dr. Rowley has stated during this meeting. The chromosome analysis from these patients substantiate the pathogenetic relationship between CMGM and CGL. Indeed, this shows remarkably fewer Ph^1-positive patients in our material compared with standard findings. This result may probably be explained by our method of obtaining the specimens: this was done after extracting the core from the site of biopsy and not be squeezing the core itself. But the corresponding values of this marker chromosome between the CGL and CMGM-groups point toward the same pathogenetic pathway.

The terms myeloid metaplasia, myelofibrosis and osteomyelosclerosis (reviews by Gralnick et al., 1971; Rappaport, 1966; Ward and Block, 1971; Burkhardt, 1971; Lennert et al., 1975) and also the one of agnogenic myeloid metaplasia (Ward and Block, 1971) are enclosed in our conception of stages III and IV from CMGM (Fig. 2d). The terms hyperplastic panmyelopathy (K. Rohr, 1960, quoted from Lennert et al., 1975), panmyelopathy (Fischer and Schäfer, 1971) or panhyperplasia (Ward and Block, 1971) is covered by our definitions of stages I and II of CMGM (Figs. 1b, 2a). – However, here is no space to enter into a more detailed discussion concerning the relationships between myeloproliferative diseases with a secondary on the one and the so called primary myelofibrosis on the other side (Fig. 2d, versus 2b). If our suggestion is correct that there is a specific pathogenic pathway leading from chronic myelogenous diseases with mixed cellularity, i.e. CMGM, to myelofibrosis then it is only reasonable to summarize and head these terms under the four stages of one disease – CMGM.

In addition clinical findings extend and confirm our morphological results mentioned above. There are different courses and different complications of both these entities: there is a faster and inevidently leukemic course in CGL complicated by bleedings and blastic crises, not usually by myelofibroses. In CMGM there is a slow, inapparent, often a- or subleukemic course which can be complicated by thromboses and even by blastic crises, and is always terminated by myelofibroses. It is of great interest to investigate whether the lymphoid blastic crises are related to the CMGM's since histopathology frequently shows lymphonoduli in the bone marrow, while this can not be detected in CGLs.

Summary

Among the patients with chronic myeloproliferative diseases including clinical symptoms of chronic myelogenous leukemia – CML – two varying compartments with substantially differing histology of hemopoiesis were found: one with predominating granulopoiesis for which the usual term of

chronic granulocytic leukemia – CGL – seems adequate. The other with proliferation of granulopoiesis and megakaryopoiesis as a neoplasia with a mixed cellularity is observed to be different in its clinical course: there are often a leukemic or subleukemic cell counts, but mostly considerable increased platelets in the peripheral blood; there is a prolonged period of latency, a higher age group, an infrequent occurrence of blastic crisis and a regular outcome into myelofibrosis. This entity of chronic megakaryocytic granulocytic myelosis – CMGM – can be seen very frequently among myeloproliferative diseases. Among a total of 718 core biopsies from the bone marrow the CMGM-patients are up to 29% compared with 21% of the typical one-cell-line disease CGL. The Ph^1-chromosome may be presented in the CMGM-entity likewise.

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