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Histology and Histopathology

Invited Review

Bone marrow histopathology in chronic myelogenous leukemia (CML)- evaluation of distinctive features with clinical impact

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Summary. Bone marrow features in stable-phase chronic myelogenous leukemia (CML) are characterized by a striking heterogeneity which is determinable by appropriate means including representative pre-treatment trephine biopsies, immunohistochemistry and morphometry. Cell lineages involved to a variable extent consist not only of neutrophil granulopoiesis, but include also megakaryocytes, erythroid precursors, resident macrophages and lymphocytes. Moreover, the stromal compartment, in particular reticulin and collagen fibers, plays a pivotal role in the disease process. Following morphometric analysis significant correlations may be calculated between histological parameters and clinical-laboratory findings. Relevant interactions are detectable between number of megakaryocytes and their precursors with fiber density. This finding is in line with the close functional relationships between megakaryopoiesis and fibroblasts regarding the complex pathomechanisms of myelofibrosis. Moreover, other correlations are observable between reduction of erythropoiesis or increase in fibers with clinical features like anemia, percentages of myelo- and erythroblasts in the peripheral blood, spleen size or LDH level. These variables are in keeping with more advanced stages of CML which indicate a transition to myeloid metaplasia and thus exert a significant impact on survival. Consequently, the different risk profiles of patients are determined by both clinical and morphological parameters of predictive value. Regarding the latter, extent of myelofibrosis, amount of erythroid precursors and numbers of myelo-erythroblasts in the peripheral blood are significantly associated with prognosis. For this reason, it should be mandatory to enter morphological criteria into prospective clinical trials on CML, not only for diagnotic purpose, but also for a proper evaluation of different survival patterns.

Key words: CML, Histopathology, Immunohistochemistry, Morphometry, Prognosis

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Introduction

Amongst the chronic myeloproliferative disorders (CMPDs) Philadelphia chromosome-positive (Ph^{1+}) chronic myelogenous leukemia (CML) is not only the most frequent subtype, but according to intensive molecularbiological investigations and comprehensive clinical trials has become the best-characterized entity (Kantarjian et al., 1988, 1993; Cline et al., 1992; Dickstein and Vardiman, 1995; Spiers, 1995; Giralt et al., 1995; Clarkson et al., 1997). This important progress in knowledge which has been achieved predominantly during the last decade may be responsible for the false impression that additional studies involving, in particular, morphological features are obsolete. Although there is still some disagreement in the literature concerning the place of the bone marrow biopsy for diagnosis of CML, the increasing use of this technique has stimulated more systematically conducted efforts to analyse the undisturbed hematopoietic tissue in situ. Following pioneering studies (Georgii et al., 1980a,b, 1990; Burkhardt and Bartl, 1982; Burkhardt et al., 1984; Lorand-Metze et al., 1987), a striking heterogeneity of bone marrow pathology has been recognized as a common finding in newly diagnosed CML. On the other hand, the exact incidence and clinical implication of this phenomenon is not well documented (Dickstein and Vardiman, 1993). In the light of considerable progress in treatment modalities like interferon (Alimena et al., 1988; Morra et al., 1992; Niederle et al., 1993; Ozer et al., 1993; Hehlmann et al., 1994, 1997; Aulitzky et al., 1995; Kantarjian et al., 1995; Ohnishi et al., 1995; Shepard et al., 1996; Richards, 1997; Guilhot et al., 1997) or bone marrow and stem cell transplantation procedures (Gratwohl et al., 1993; McGlave et al., 1994; Appelbaum et al., 1995; Van Rhee et al., 1997; Clift and Anasetti, 1997; Gale et al., 1998) more attention is being paid to prognostic models and risk factors. These calculations were aimed at a classification of patients into certain groups characterized by different survival patterns (Cervantes and Rozman, 1982; Sokal et al., 1984, 1988; Kantarjian et al., 1985; The Italian Study Group, 1991; Hasford et al., 1996, 1998; Cortes et al.,

1997). Until now, development of staging systems was focused on clinical variables (Kantarjian et al., 1990) and, as should be explicitly pointed out, largely failed to consider morphological parameters. Regarding these short-comings, several authors even discussed whether the introduction of a histological classification scheme was superflous and therefore put into doubt the clinical interest of any subtyping based on histopathology (Knox et al., 1984; Cervantes et al., 1989; Rozman et al., 1989). On the other hand, it may be argued that determination of morphological parameters suspected to exert a prognostic impact has to be preceded by a definitive identification of all the various cell lineages and distinctive structures composing the complex architecture of bone marow tissue in CML. Major marrow constituents encompass not only the clonally transformed predominant cell lineages, but also the stromal (mesenchymal) compartment harboring both leukemic and non-clonally proliferating cells and connective tissue components (Mayani et al., 1990) which are consistent with the (extracellular) micro-environmental matrix (Table 1). These considerations are motivating points that warrant a refined assessment by applying enzyme and immunohistochemical techniques and morphometric quantification. Since this problem remains an open and controversial issue, we have reviewed the pertinent literature in this field and re-evaluated our filed specimens together with the corresponding clinical records. In this context histochemical methods included naphthol-AS-D-chloroacetate esterase staining, Perl's reaction and PAS (periodic acid Schiff reagent) and a number of monoclonal antibodies for the identification of the different cell lineages: CD61-megakaryopoiesis and Ret 40 f - erythroid precursors (Gatter et al., 1988); CD68 - macrophages (Falini et al., 1993); BSA-I lectin (Maddox et al., 1982; Irimura et al., 1987); CD20; and CD45RO for the B- and T-lymphocyte subsets (Knapp et al., 1989).

Histopathology - overview

Histological features that are commonly exhibited in biopsy specimens of patients presenting stable-phase CML include an overall hypercellularity with effacement of the age-related adipose tissue by a predominant growth of neutrophil granulopoiesis (Fig. 1a) and reduction in erythropoietic islets (Fig. 1b,c). These changes may be accompanied by varying numbers of megakaryocytes (Fig. 2a,b) and amount of reticulin fibers (Fig. 3a,b). Granulocytic proliferation consists of broad endosteal (paratrabecular) and perivascular seams of immature precursors revealing a stepwise

Bone marrow features

parenchymal — **Compartment** — mesenchymal

clonally transformed (leukemic) hematopoiesis		*non-clonally proliferating constituents*
Granulopoiesis	Macrophages and subpopulations	Fibroblasts /- cytes
Megakaryopoiesis	Plasma cells	Endothelial cells - vascular structures
Erythropoiesis	Mast cells (?)	Adipocytes
	B-lymphocytes (?)	T-lymphocytes (?)
		(Reticulin and collagen fibers, - types I,III,IV)
		Fibronectin - Laminin extracellular matrix)
Predominant hematopoietic cell lineages responsible for diagnostic clinical-laboratory findings	**Myeloid stroma** (microenvironment) consisting of a very complex functional, cytokine-mediated network between cells and interstitial structures	

Table 1. Structural and functional composition of the bone marrow in CML.

differentiation into polymorphonuclear terminal forms in the central marrow region (Burkhardt et al., 1984; Georgii et al., 1990; Thiele et al., 1990a). On the other hand, in comparison to the normal bone marrow, megakaryocytes are characterized by their small, dwarf-like appearance containing hypolobulated dense nuclei (Figs. 3c, 4c), so-called atypical micromegakaryocytes (Burkhardt et al., 1990; Thiele et al., 1990b; Thiele and Fischer, 1991; Nafe et al., 1991; Buhr et al., 1992; Bartl et al., 1993). Myelofibrosis is generally defined as an increased deposit of reticulin and/or collagen fibers in the extracellular (interstitial) space and terminally may merge into endophytic primitive bone formation (osteosclerosis). Initial stages include a perivascular arrangement of fine reticulin fibers (sinus wall sclerosis) which at later stages usually transform into a coarse network in close proximity to megakaryocyte clusters (Fig. 3a,b) and finally may reveal an extention to widespread thick collagen bundles (terminal fibrosclerotic stage). Onset of blastic crisis, i.e. instable-accelerated phase of CML (Muehleck et al., 1984) is indicated by an increase in the endosteal seams of precursors revealing a more than five-row-deep accumulation of myeloblasts-promyelocytes (Georgii et al., 1990). Characteristically there is no stepwise maturation into polymorphonuclear granulocytes in the intermediate-central marrow spaces causing a hiatus-like arrest of maturation. Manifest blastic crisis is characterized by a total effacement of differentiation of the granulocytic lineage revealing an overall growth of primitive precursors and thus resembles acute myeloblastic leukemia.

Histochemistry and morphometry

General considerations

With accumulating experience it becomes more and more evident that new insights into hematopoietic

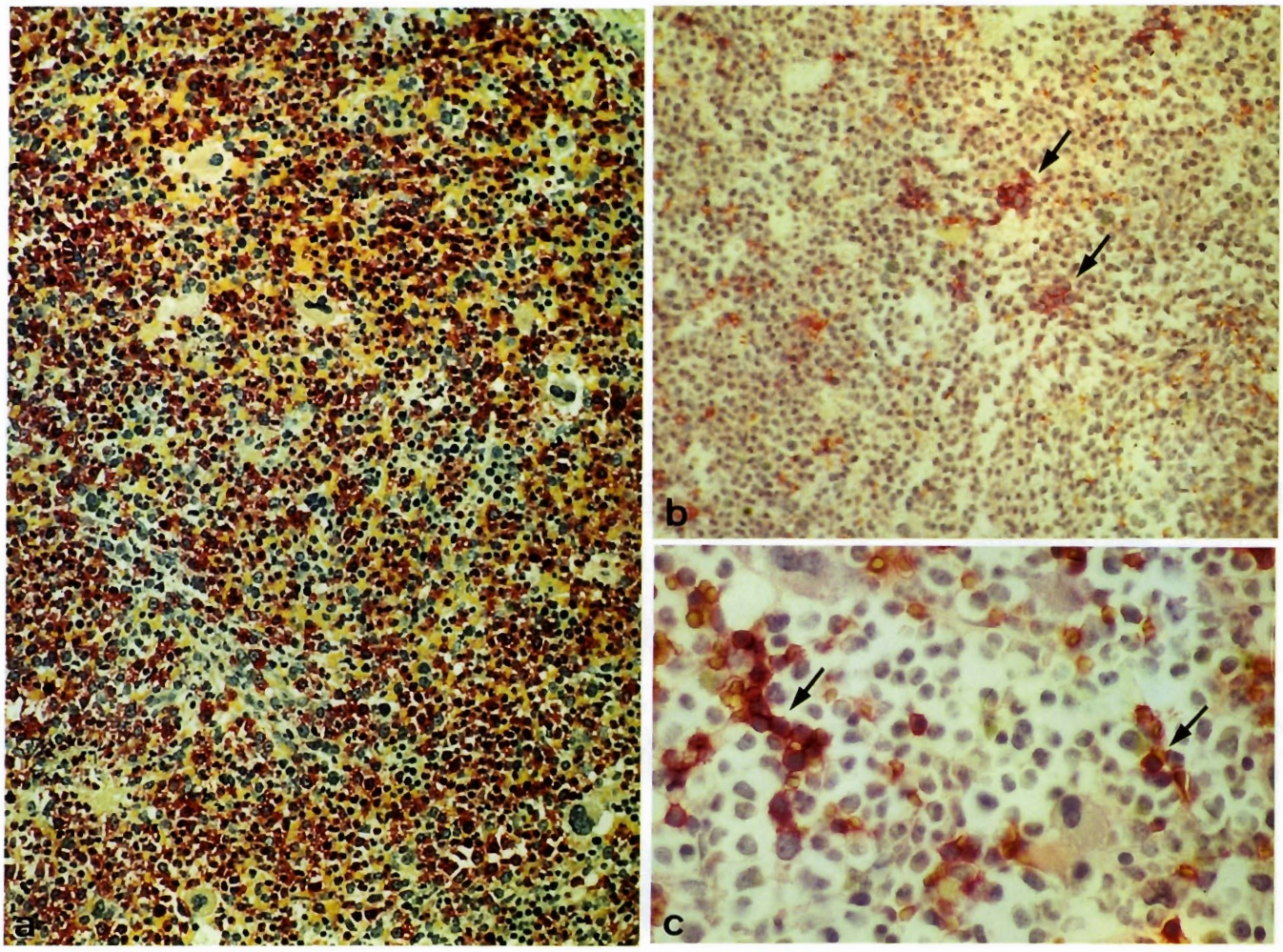

Fig. 1. Neutrophil granulopoiesies and erythropoiesis in CML bone marrow. **a.** Predominant growth of naphthol-AS-D-chloroacetate esterase-positive granulopoiesis with total reduction of the age-related adipose tissue. **b.** Significant decrease in the number of erythroid islets (arrows) which consist of **c.** tiny groupings of small erythroblasts (arrows). a and b, x 170; c, x 370

pathology may be gained by histochemical labelling followed by morphometric quantification of the different cell lineages, as shown in Table 2. This procedure furthers the understanding of the complex composition of bone marrow tissue in CML (Table 1), more than may be achieved by conventional staining or the employment

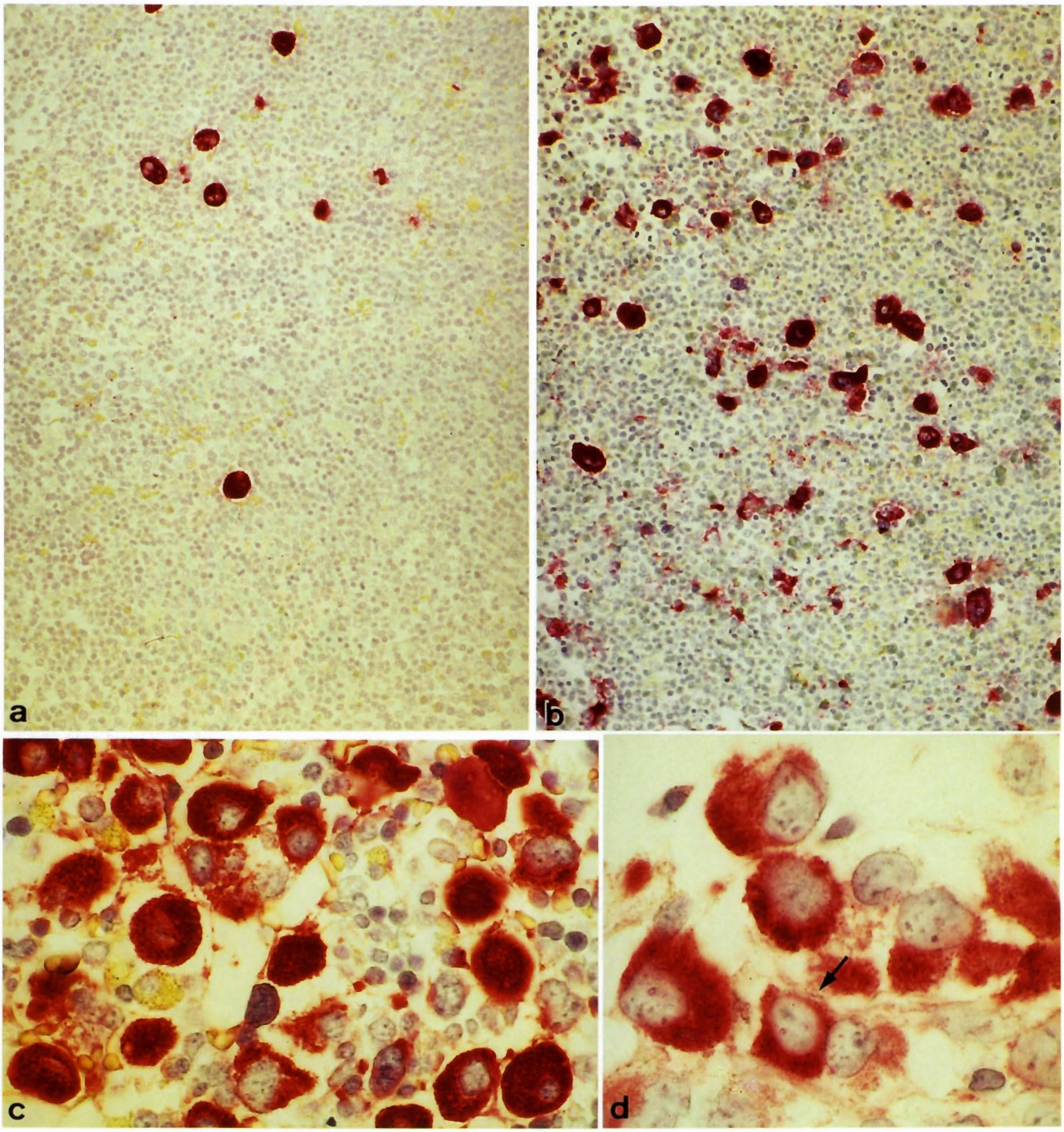

Fig. 2. Megakaryopoiesis in CML bone marrow. **a and b.** CD 61+ megakaryocytes show significant differences in frequency and many microforms. **c.** Assembly of atypical micromegakaryocytes with hypolobulated nuclei. **d.** Immature elements of megakaryopoiesis consistent with megakaryoblasts and a promegakaryoblast (arrow). a and b, x 170; c, x 570; d, 1,270

of aspirate material. Conspicuously wide ranges of frequency were encountered, when considering erythroid precursors, megakaryocytes and fiber density (Table 2). This striking heterogeneity of bone marrow features in CML which may be only incompletely assessed in smears, implicates a corresponding variability of clinical findings at presentation of patients (Spiers, 1995; Dickstein and Vardiman, 1995; Cortes et al., 1997). For instance, the significant reduction of erythroid precursors is readily demonstrable together with a corresponding shift of the ratio between the granulocyte/erythroid compartment (Fig. 1a-c). Although anemia or the hemoglobin level were determined as one of the most distinctive factors exerting a prognostic impact (Cervantes and Rozman, 1982; Kantarjian et al., 1985; Sokal et al., 1988; The Italian Cooperative Study Group, 1993), a quantitative analysis of erythroid precursors in the CML bone marrow has rarely been performed (Thiele et al., 1993b, 1995). Furthermore, a more elaborate evaluation of megakaryopoiesis, an assessment of (reticulin) fiber density, the determination of the resident macrophages including their various subpopulations and finally the B- and T- lymphocytes becomes feasible.

Megakaryocytes and fibers

In addition to the mature megakaryocytes with their well-recognized microforms in CML (Fig. 4c) which may be identified by Giemsa, PAS or preferentially by CD61 staining (Thiele et al., 1988, 1990a; Burkhardt et al., 1990; Georgii et al., 1990; Buhr et al., 1992; Bartl et al., 1993), a considerable number (about 30%) of very small abnormal and immature precursors (Fig. 2c,d) are present which may easily escape recognition (Thiele et al., 1990b; Thiele and Fischer, 1991). The amount of this peculiar $CD61^+$ cell population may be assessed not only by regarding its cytological appearance (Fig. 2d), but also by its failure to react with PAS (Thiele et al., 1990a,b). This compartment of megakaryocyte

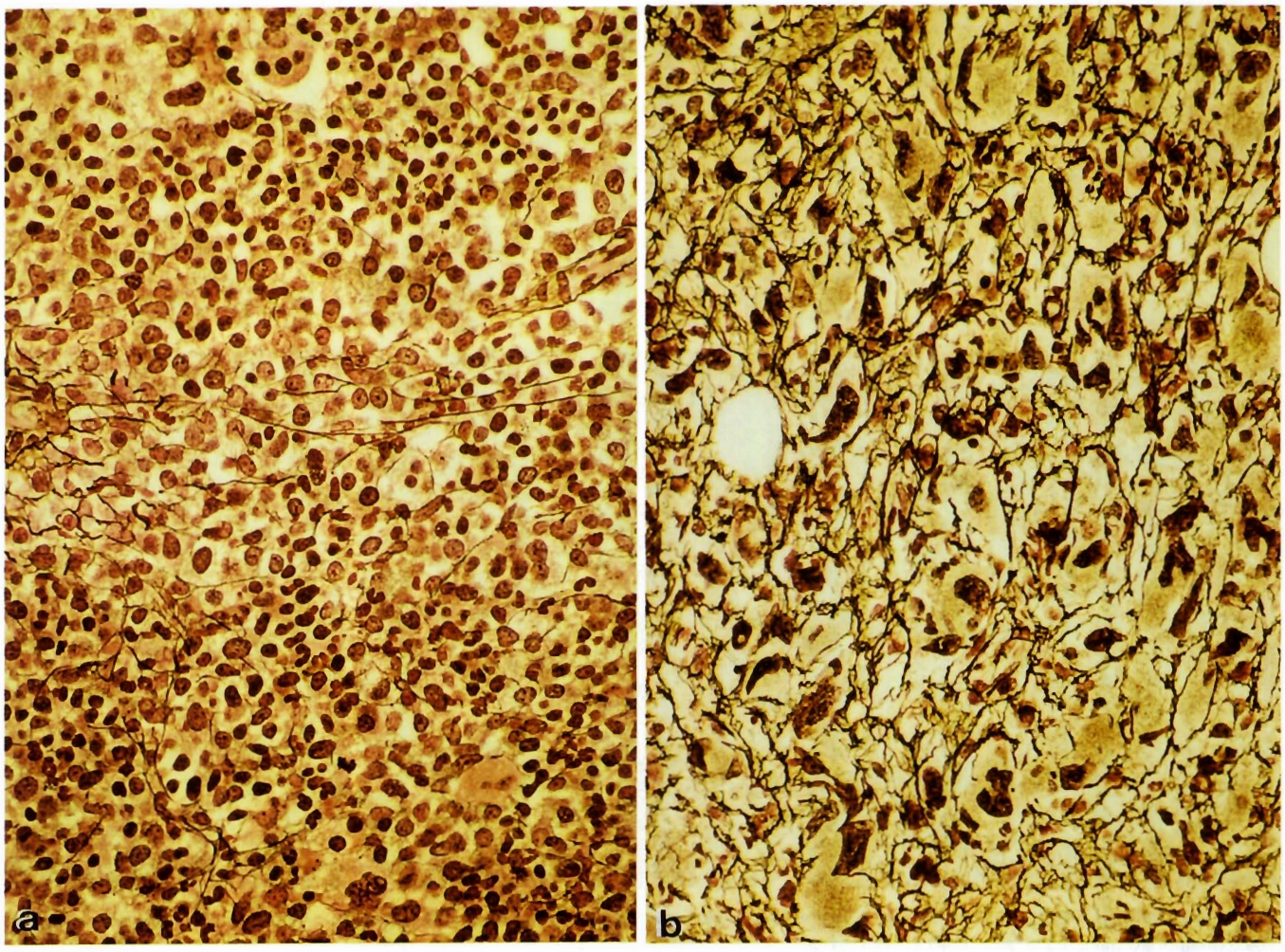

Fig. 3. Fibers in CML bone marrow. **a.** Borderline increase in finely dispersed reticulin fibers. **b.** Coarse collagen fibers in close association with abnormal megakaryocytes following silver impregnation. x 370

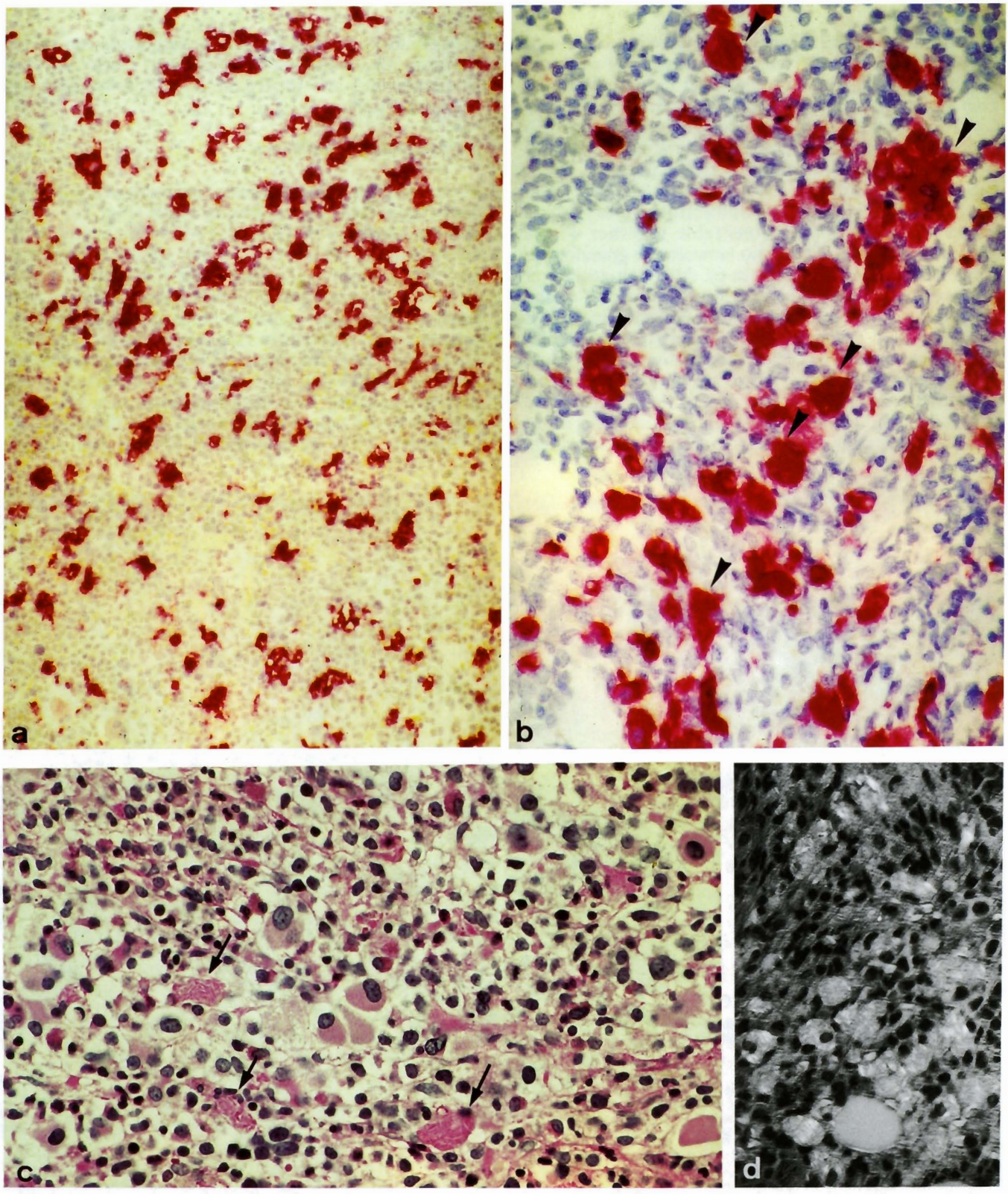

Fig. 4. Macrophages in CML bone marrow. **a.** CD68$^+$ mature resident macrophages reveal a stellate irregular shape. **b.** Activated subset of BSA-I$^+$ macrophages including many large Pseudo-Gaucher cells (arrow heads). **c.** Scattered PAS$^+$ Pseudo-Gaucher cells (arrows) display an onion skin-like pattern and are easily distinguishable from micromegakaryocytes (center) with their dense hypolobulated nuclei. **d.** Positive birefringence of clustered Giemsa-stained Pseudo-Gaucher cells following polarization. a, x 170; b-d, x 370

Table 2. Histochemical and morphometric evaluation of bone marrow pathology on pretreatment biopsies in about 500 patients with Ph^{1+}-CML. Values were determined per mm^2 bone marrow area with a hematopoietic cellularity at 98.1±2.5% in CML compared to age-matched controls at 47.3±12%.

PARAMETER	CML mean±SD (range)	CONTROLS mean±SD (range)
Neutrophil granulopoiesis x 10^2		
(naphthol-AS-D-chloroacetate esterase)	95±35 (59-135)	35±7 (28-46)
Erythroid precursors x 10^2		
(antiglycophorin C)	4.5±3.2 (2.9-18.4)	11.2±3.1 (7.2-19.5)
Megakaryopoiesis		
total $CD61^+$	85±82 (3-745)	24±6 (14-34)
mature PAS^+	55±54 (2-405)	15±3 (10-23)
Macrophages x 10^2		
total $CD68^+$	3.0±0.9 (0.8-5.9)	3.7±0.9 (3.1±4.2)
activated BSA-I^+	1.7±0.5 (0.7-3.9)	2.0±0.7 (1.6±2.4)
Pseudo-Gaucher cells (%)	31	-
Iron-laden subset (%)*	39	95
Lymphocytes		
diffuse infiltrates		
B-Lymphocytes $CD20^+$	4.7±4.6 (1-29)	4.6±1.0 (3-6)
T-Lymphocytes $CD45R0^+$	18.5±13.1 (2-46)	15.4±8.2 (9-32)
lymphoid nodules (%)	4.25	-
Argyrophilic fibers i x 10^2 **		
(Gomoris's silver impregnation method)	39±23 (5-146)	16±5 (4-20)

*: Amount of stainable iron is significantly to moderately reduced in this fraction revealing a normal content in only 2.5%. **: Density of argyrophilic (reticulin-collagen) fibers was measured as intersections (i) with an ocular grid at x500 magnification per hematopoiesis (cellularity).

precursors has been found to play a pivotal role in the generation of myelofibrosis (Terui et al., 1990; Reilly et al., 1993; Thiele et al., 1997). Megakaryoblasts produce and secrete an activated form of transforming growth factor- ß (TGF-ß) and thus stimulate collagen synthesis in the non-clonally-transformed bone marrow fibroblasts (O'Brien et al., 1988) in a paracrine manner (Kimura et al., 1995; Martyré, 1995; Reilly, 1992). In confirmation and extention of this currently accepted hypothesis on the pathogenesis of myelofibrosis in CMPDs, a significant correlation not only between the total number of mature megakaryocytes (Lazzarino et al., 1986; Buhr et al., 1992; Thiele et al., 1997), but more important also its immature (PAS^- and $CD61^+$) fraction (Fig. 2d) has been observed (Thiele et al., 1993a). In this context, histopathology provided the first tangible evidence for a stepwise evolution of medullary fibrosis associated with an increased growth of abnormal megakaryocytes (Buhr et al., 1992; Thiele et al., 1993a, 1997).

Macrophages

Resident macrophages of the bone marrow constitute a strikingly heterogeneous population that can be divided into several subsets (Table 2). Regarding the $CD68^+$ mature macrophage compartment (Fig. 4a) no significant difference in frequency compared to the normal bone marrow may be encountered (Table 2). Various in-vitro studies involving macrophage populations from rodents are in keeping with the assumption that lectins detecting α-D-galactosyl residues like *Bandeiraea (Griffonia) simplicifolia* isotype I-B_4 (BSA-I) identify activation-associated antigens and therefore corresponding macrophage subpopulations (Maddox et al., 1982; Adams and Hamilton, 1984; Irimura et al., 1987; Tabor et al., 1989; Keller et al., 1993; Warfel and Zucker-Franklin, 1993). Previous studies (Baldus et al., 1995) have reported that between 50 to 60% of the $CD68^+$ macrophages of the normal bone marrow express this carbohydrate binding pattern (BSA-I^+). In CML no significant quantitative alteration in the fraction of BSA-I^+ macrophages (Fig. 4b) has been found (Table 2). According to their myelomonocytic progency and following the detection of the Philadelphia chromosome (Golde et al., 1977) or bcr/abl translocation (Thiele et al., 1998b), interest has been recently reawakened in this cell lineage (Bhatia et al., 1991; Thiele et al., 1992). The abnormal function of the CML bone marrow, in particular the disturbance of stem cell differentiation and maturation has been related to a defective adhesion of progenitors to stroma cells (Dowding et al., 1993). Several laboratories have demonstrated distinctive changes in the adhesive properties of CML progenitors to the marrow microenvironment (Galvani and Cawley, 1990; Dowding et al., 1991; Osterholz et al., 1991). Normal primitive progenitors adhere well to the different constituents composing the marrow stroma (Table 1) and are usually quiescent when in close contact with these structures (Obinata et al., 1998). Contrasting to this commonly expressed homing phenomenon, CML progenitors display a significantly reduced adherence to the normal stroma marrow layers and are continuously proliferating (Bhatia and Verfaille, 1998). For this reason, in the context of interferon treatment it has been discussed whether and to what extent clonally-transformed (leukemic) macrophages may contribute to this obvious microenvironmental dysfunction. Currently it is speculated that macrophages play a prominent part in the selective expansion of the neoplastic cell clone followed by a suppression of normal hematopoiesis (Bhatia et al., 1991; Dowding et al., 1993; Santucci et al., 1993; Straetmans and Ma, 1996). A conflict of opinion arises regarding another sub-population of activated BSA-I^+ macrophages, the Pseudo-Gaucher cells (Fig. 4c,d). Controversial findings have been expressed concerning their incidence in the CML bone marrow which was reported to range between 20 to 70% (Buesche et al., 1997). Based on our evaluation, which includes a more

elaborate identification process demanding not only positive fibrillar birefringence following polarization of Giemsa-stained slides (Fig. 4c), but also a positive reaction with PAS and BSA-I, the frequency is about 30% (Table 2). Pseudo-Gaucher cells may aid in the differentiation of CML from severe leukemoid reaction (Schmidt et al., 1990). The presence of this subset of BSA-I$^+$ macrophages including also the so-called sea-blue histiocytes has been widely linked to an increased cell turnover with enhanced desintegration of the clonally-transformed leukemic cell mass (Hayhoe et al., 1979). Therefore, putative prognostic properties have been linked to the presence of Pseudo-Gaucher cells (Albrecht, 1972; Kelsey and Geary, 1988; Thiele et al., 1990a, 1991, 1993a). On the other hand, some restrictions concerning this finding should not be overlooked, since data were derived from small series of patients and thus warrant further support and prospective studies. Finally, another subpopulation of macrophages deserves attention, i.e. the hemosiderin/ferritin-laden histiocytic reticular cells which are closely associated with the iron metabolism of erythropoiesis. In keeping with our results of a significantly reduced specific staining capacity (Table 2), about 80% of patients with CML were reported to have no or only a minimal amount of marrow iron at time of diagnosis (Cervantes

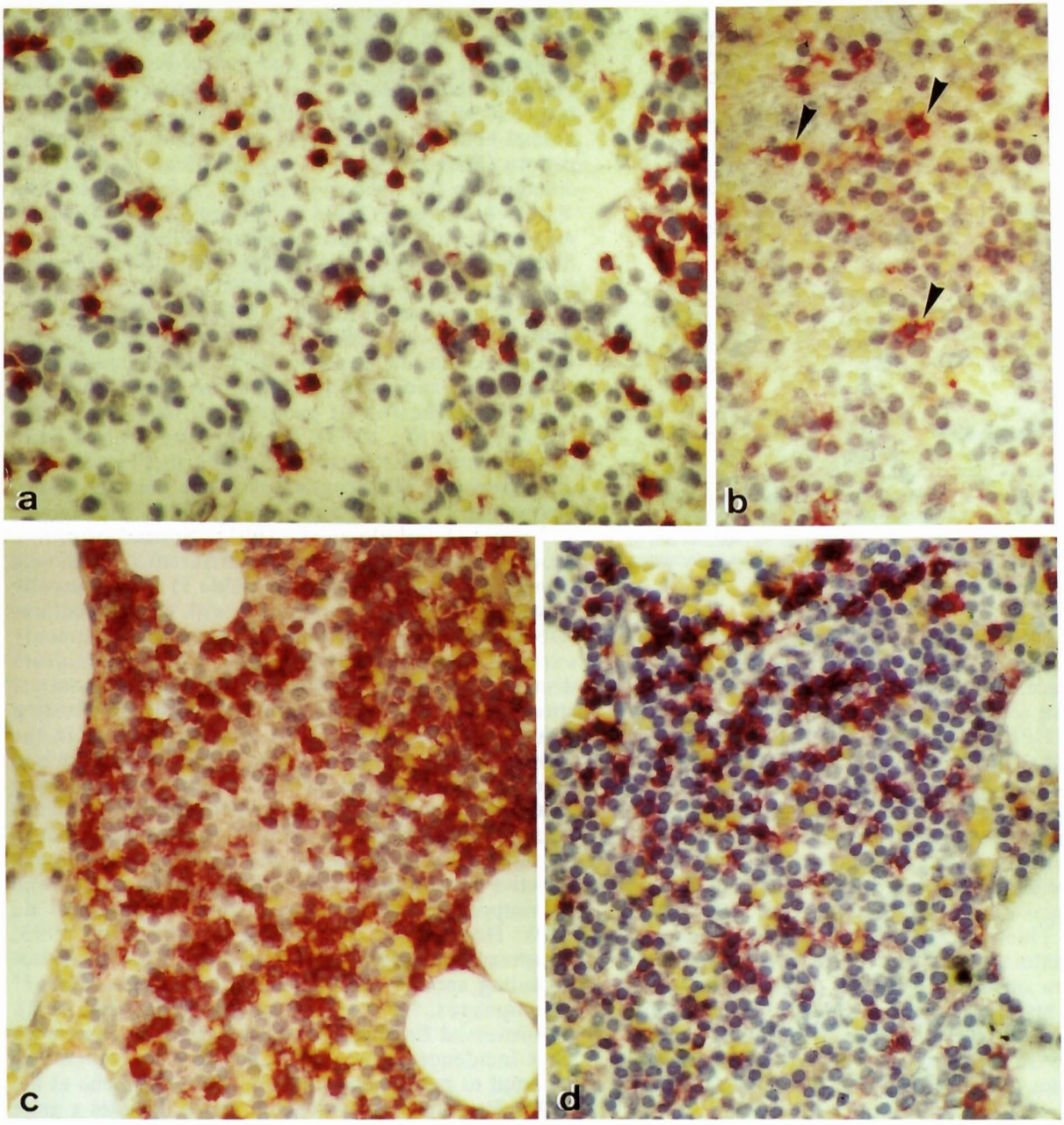

Fig. 5. Lymphocytes and lymphoid nodules in CML bone marrow. Lymphocytes show a predominance of **(a)** T-(CD45RO$^+$) and a small number of scattered **(b)** B-(CD20$^+$) lymphocytes (arrow heads). Lymphoid aggregates are mostly composed of CD45R0$^+$ lymphocytes **(c)** with admixture of few CD20$^+$ lymphocytes **(d)**. x 370

et al., 1984, Sokal and Sheerin, 1986). On the other hand, these findings did not reflect the iron status, since CML patients have normal serum iron, total iron binding capacity and serum ferritin levels (Cervantes et al., 1986). Noteworthy is that iron deposits and number of sideroblasts increased when patients presented with or evolved into an accelerated-blastic phase and consequently a careful determination of marrow iron was discussed as a useful prognostic indicator for the disease state in CML (Welborn and Lewis, 1993).

Lymphocytes

Lymphoid cells in the CML bone marrow occur either as diffuse, loosely scattered infiltrates (Fig. 5a,b) or rarely are assembled focally, so-called lymphoid nodules (Fig. 5c,d). With respect to the clonal nature of CML the extent of lineage involvement is a matter of interest, because the lymphoid lineage diverges from the myeloid lineage at an early stage of hematopoietic differentiation (Haferlach et al., 1997). Controversy and discussion arise regarding the presence of the Philadelphia chromosome or the bcr/abl gene translocation in this cell population (peripheral blood or bone marrow). Direct evidence of involvement of T- (Nogueira-Costa et al., 1986; Jonas et al., 1992; Tsukamoto et al., 1996; Haferlach et al., 1997) or B-lymphocytes (Martin et al., 1980; Bernheim et al., 1981; Nitta et al., 1985; Bartram et al., 1987; Ferraris et al., 1989; Garicochea et al., 1994; Torlakovic et al., 1994 Haferlach et al., 1997) in CML is inconclusive (MacKinney et al., 1993; Tefferi et al., 1995; Haferlach et al., 1997). Following cell culture experiments (Nogueira-Costa et al., 1986) and cytogenetic and molecularbiological studies (Bartram et al., 1987; Garicochea et al., 1994) compelling evidence has been produced to show that the majority of T-lymphocytes are polyclonal during the stable phase of CML (Tsukamoto et al., 1996). Despite various clinical trials the presence and biological implication of nodular or scattered B- and T-lymphoid infiltrates in the bone marrow of CML patients has not been explained in detail and until now no quantitative data have been available. Following a recently conducted immunohistochemical identification and morphometric analysis of the B- and T-lymphoid cell population, the CML bone marrow revealed no significant difference in comparison to control specimens (Table 2). With respect to the other CMPDs the incidence of lymphoid nodules (Fig. 5c,d) was reported to range between 2 to 12% in CML (Hernández-Nieto et al., 1979; Frisch et al., 1984; Navone et al., 1985; Cervantes et al., 1988; Franco et al., 1991) and therefore is less frequent than in the other subtypes (Table 2). Immunohistopathological studies revealed that these aggregates were composed of variable fractions of both B ($CD20^+$)- and T ($CD45RO^+$)-lymphocytes with a prevalence of T-cells (Franco et al., 1991). The higher incidence of the lymphoid nodules in CMPD subtypes accompanied by myelofibrosis suggests that their presence could be related to interactions between stromal microenvironment and immunological processes associated with the complex pathomechanism of fibrillogenesis. The latter could either involve the autoimmune system or an activation of the complement system, as has been speculated for idiopathic (primary) myelofibrosis - agnogenic myeloid metaplasia (Caligaris-Cappio et al., 1981; Gordon et al., 1981; Rondeau et al., 1993). An interaction of immune complexes with the myelofibrosis-promoting growth factor release by the megakaryo-thrombocyte lineage (Reilly, 1992; Kimura et al., 1995, Martyre, 1995) may also be hypothesized. However, our immunohistological identification of the T and B-lymphocyte subsets followed by morphometric evaluation (Table 2) failed to show any correlation with the degree of reticulin fibrosis.

Table 3. Significant correlations between myelofibrosis and erythroid precursors in the bone marrow with histological parameters in Ph^{1+}-CML investigated in a cohort of about 500 patients.

PARAMETER	FIBERS	ERYTHROID PRECURSORS
Fiber density		- 0.1473*
Megakaryocytes		
Total	0.2909*	-
Precursors	0.2979*	-
Macrophages		
Total	-	0.2583*
"Activated" subpopulation	-	0.2598*

Level of significance:* $p \leq 0.001$

Interactions of morphological variables

A number of significant correlations between histological parameters and laboratory findings imply various functional relationships (Thiele et al., 1990a, 1993a). In previous studies these calculations have rarely been carried out. This shortcoming is probably due to the general impairment of semiquantitative grading and the difficulty either to assign histological subtypes to established clinical entities, i.e. CML (Burkhardt et al., 1986) or to avoid the erroneous inclusion of so-called atypical forms and chronic myelomonocytic leukemia (Bennett et al., 1997; Costello et al., 1997). Significant differences ($p<0.001$) concerning the incidence of $CD61^+$ megakaryocytes were calculable, when comparing patients presenting a normal platelet count ($\leq 350 \times 10^9/l$) to another group with thrombocytosis exceeding $600 \times 10^9/l$ (Thiele et al., 1999). Strikingly, adverse interactions were further determinable between number of bone marrow erythroid precursors versus fiber density (Table 3). Moreover, the positive correlations of macrophages including their activated subset with the amount of erythroid precursors are in keeping with the well-known functional involvement of this cell population in iron turnover and hemoglobin

Table 4. Significant correlations ($p \leq 0.05$) between numbers of erythroid precursors with clinical parameters in $Ph1^+$-CML according to two groups of patients (cut off point at median value). The erythroid cell count in normal bone marrow is 1,123±312mm^2.

HEMATOLOGICAL PARAMETERS	ERYTHROID PRECURSORS GROUP I <370/mm^2	GROUP II ≥ 370/mm^2	P
Hemoglobin (g/dl)	11.8±2.2	12.5±2.1	0.004
Hematocrit (%)	37.1±9.2	39.1±6.2	0.036
Erythrocytes (x10^{12}/l)	4.00±0.73	4.30±0.72	0.001
Leukocytes (x10^9/l)	143±115	76±77	0.000
Polymorphonuclear granulocytes (%)	42.9±15.6	49.3±16.0	0.000
Promyelocytes (%)	5.2±6.4	2.9±4.5	0.000
Myeloblasts (%)	3.2±5.2	1.4±2.6	0.000
Basophils (%)	4.2±4.5	3 .1±3.6	0.012
LDH (U/L)[a]	704±344	546±306	0.000
LAP (score)[b]	10±18	17±33	0.019
Spleen size (cm)[c]	4.1±4.7	2.2±3.1	0.000
Liver size (cm)[c]	1.3±2.4	0.8±2.0	0.024

[a]: lactat-dehydrogenase; [b]: leukocyte alkaline phosphatase (normal range 20-80); [c]: spleen/liver size: cm below costal margin.

Table 5. Significant correlations ($p \leq 0.05$) between density of argyrophilic (reticulin-collagen) fibers with clinical parameters in $Ph1^+$-CML according to two groups of patients (cut off point was a three-fold increase in normal fiber density of 16±5x10^2/mm^2 in the unaltered bone marrow).

HEMATOLOGICAL PARAMETERS	FIBER DENSITY GROUP I <50ix10^2/mm^2	GROUP II ≥ 50ix10^2/mm^2	P
Hemoglobin (g/dl)	12.3±2.1	11.2±2.3	0.000
Hematocrit (%)	38.4±70.0	34.9±6.9	0.000
Erythrocytes (x10^{12}/l)	4.2±0.7	3.8±0.7	0.000
Leukocytes (x10^9/l)	104±98	141±63	0.002
Polymorphonuclear granulocytes (%)	47.2±16.1	42.7±15.3	0.009
Myeloblasts (%)	1.8±3.3	3.5±6.9	0.015
Basophils (%)	3.4±3.3	4.7±5.4	0.026
LDH (U/L)	609±322	771±413	0.001
Spleen size (cm)	2.8±3.8	4.6±4.7	0.001

synthesis. With regard to clinical features significant differences may be calculated when generating two groups of patients: one with an erythroid precursor count less than the median value of 370/mm^2 (group I), which amounts approximately one forth the normal bone marrow frequency (Thiele et al., 1993b) and another cohort exceeding this cut-off point (group II). Table 4 shows that laboratory data of group I are characterized by increasing anemia, leukocytosis, hepatosplenomegaly and myeloblasts in the peripheral blood together with a reduction of mature granulocytes. As has already been pointed out, argyrophilic (reticulin-collagen) fiber density correlates not only with the number of mature and precursor megakaryocytes (Lazzarino et al., 1986; Buhr et al., 1992; Thiele et al., 1993a, 1997), but also with a variety of clinical features. This may be demonstrated when dividing the total cohort of CML patients into one without relevant fiber increase (fiber density <50 i x 10^2/mm^2) corresponding with group I and another with early to advanced myelofibrosis (fiber density ≥ 50 i x 10^2/mm^2) consistent with group II (Table 5). These associations relating to reduction of erythropoiesis or generation of myelofibrosis and ensuing clinical features (anemia, increase in the number of myeloblasts and spleen size) are in keeping with more advanced stages of CML indicative for a transition into myeloid metaplasia. Consequently, it may be speculated that these interrelationships play a key role in the duration of stable-phase CML and thus may exert a significant impact on survival.

Prognostic implications

The wealth of data accumulated in the past decade about prognostic features in CML has been significantly hampered by the obvious failure to enter morphological parameters into the generally accepted models for calculation of survival (Kantarjian et al., 1990; Hasford et al., 1996, 1998; Cortes et al., 1997). There is great disagreement as to whether pretreatment discrimination of patients into different risk groups is feasible by evaluating bone marrow morphology (Hehlmann et al., 1997). Several authorities have focused their attention on the presence of myelofibrosis; however, with conflicting results concerning an unfavorable (Lazzarino et al., 1986; Dekmezian et al., 1987; Thiele et al., 1988, 1991, 1993a, 1995, 1998a) or favorable influence on prognosis (Burkhardt and Bartl, 1982; Frisch and Bartl, 1985; Bartl et al., 1993). This controversial point has been repeatedly discussed also with regard to bone marrow transplantation procedures, i.e. the pretransplantant influence of myelofibrosis for engraftment failure (Rajantie et al., 1986; Soll et al., 1995). Again it has to be kept in mind, that evaluation of fiber content was performed by semiquantitative gradings using different scoring systems (Gralnick et al., 1971; Clough et al., 1979; Dekmezian et al., 1987). From these studies it was derived that only a substantial reticulin or collagen fibrosis exerts a useful predictive impact which indicates a worsening of survival (Clough et al., 1979; Dekmezian et al., 1987; Kantarjian et al., 1988). Opposed to this finding, morphometric determination of the degree of myelofibrosis revealed that even initial stages, i.e. a doubling of normal fiber density (Thiele et al., 1993a, 1998a) already resulted in a significant reduction of life expectancy (Fig. 6a). Similar findings may also be encountered when calculating the amount of erythroid precursors in the bone marrow (Fig. 6b). Conflicting statements have also been published regarding the prognostic implication of megakaryocytes. In some studies megakaryocytopenia was believed to indicate poor survival (Kantarjian et al., 1985, 1988). On the other hand, megakaryocyte-rich subtypes of the Hannover Classification (Georgii et al., 1990) were

described as signalling a later transition into myelofibrosis and therefore were assigned as prefibrotic lesions (Buhr et al., 1992). Because a significant relationship between megakaryocytes and fiber density is now well-established (Thiele et al., 1993a, 1997), increase in this cell lineage may indeed herald myelofibrosis and thus is in keeping with an unfavorable course. Besides these morphological variables, clinical risk factors include in addition to age, anemia, splenomegaly, peripheral blast count and thrombocytes (Sokal et al., 1984, 1988; Kantarjian et al., 1985, 1988, 1993; Thiele et al., 1988, 1993a, 1998a; Hasford et al., 1996, 1998; Hehlmann et al., 1997). As may be easily derived from Fig. 6c,d these features exert a significant influence on survival independently from therapeutic regimens.

Staging systems

Based on semithin sections of plastic-embedded and routinelys-tained (Giemsa, Gomori's silver impregnation) sections of core biopsies and a semiquantitative scoring system, an attempt was carried out to classify bone marrow histopathology into different categories (Burkhardt et al., 1984; Georgii et al., 1990; Buhr et al., 1992). However, dependent on the inherent inconsistency of such gradings with their differences in the quality of scoring, the results of two leading studies in this field are hardly comparable with each other (Burkhardt et al., 1984; Georgii et al., 1990). Although a so-called unilinear granulocytic type has been discriminated from a bilinear granulocytic/megakaryocytic and myelofibrotic subtype, a definitive separation of CML from the allied subtypes of CMPDs remains debatable (Burkhardt et al., 1984; Bartl et al., 1993). Intentions to correlate these histological subtypes with corresponding clinical data and survival were ambiguous (Burkhardt et al., 1986, 1990). These inconsistencies may be demonstrated for the myelofibrotic subtype. Following clinicopathological investigations from different trials, myelofibrosis is generally assumed as an

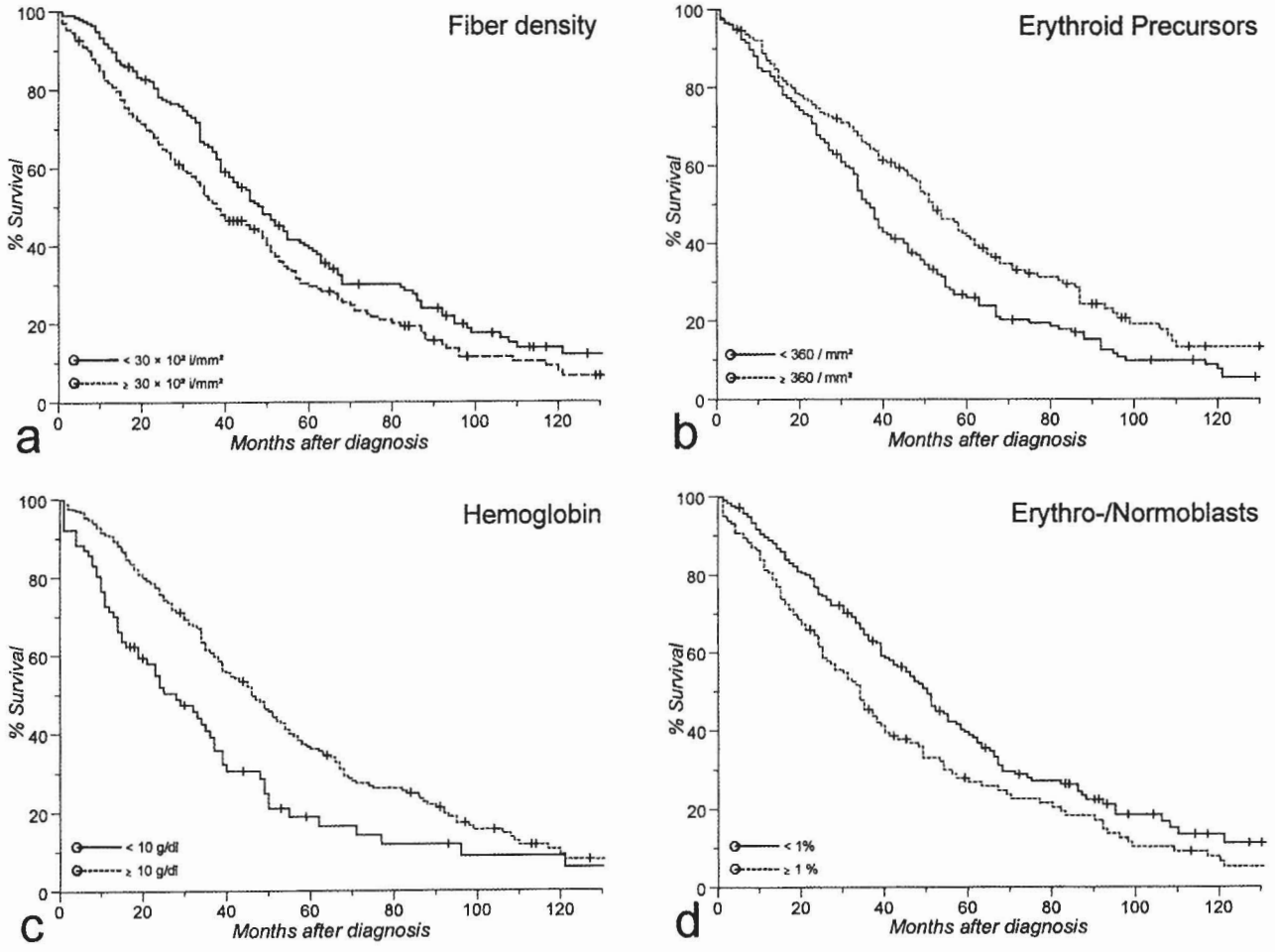

Fig. 6. Survival of patients with CML under chemotherapy (busulfan-hydroxyurea) Histological variables: **(a)** fiber density (i x 10^2/mm^2) and **(b)** erythroid precursors (mm^2). Clinical parameters: **(c)** degree of anemia - hemoglobin level (g/dl) and **(d)** erythroblasts in the peripheral blood (%).

Table 6. Main categories of the Hannover Classification of CML (Georgii et al., 1990). The intermediate or overlapping (OT) subtypes were not regarded.

CML.CT	Common or granulocytic type of CML
CML.MI	Megakaryocytic increase
CML.MP	Megakaryocytic predominance
CML.EMS	Early (reticulin) myelofibrosis
CML.MS/MF	Myelosclerosis-(collagen) myelofibrosis
CML.AMF	Advanced (collagen) myelofibrosis
CML.BC	Blastic crisis

Table 7. Cologne classification of histological subtypes in CML and correlation with clinical features.

omnious sign that heralds the transition of stable-phase CML into an accelerated phase and blastic crisis (Muehleck et al., 1983). In accordance with these results a growing body of evidence has been produced that indicates a worsening of survival in patients showing myelofibrosis (Gralnick et al., 1971; Clough et al., 1979; Lazzarino et al., 1986; Dekmezian et al., 1987; Thiele et al., 1988, 1991, 1990a, 1993a, 1995, 1998a). In the aforementioned classification system, opposed to these well-accepted findings, the myelofibrotic subtype of CML was held to decrease the risk of developing blastic crisis or an unstable phase and thus was compatible with a more favorable prognosis (Burkhardt et al., 1982; Frisch and Bartl, 1985; Bartl et al., 1993).

On the other hand, a significant progress in subtyping histological features of the CML bone marrow has been achieved by the Hannover Classification. The rationale of this staging system (Table 6) is to differentiate between primary (initial) and advanced disorders (Georgii et al., 1990). This novel approach to CML histopathology results in the discrimination of two different (primary) subtypes at onset of disease, i.e. the most frequently occurring common granulocytic subtype (CML.CT) versus another group showing an increase or predominance of megakaryocytes (CML.MI/MP). All other subtypes exhibiting either an early reticulin (CML.EMS) or more advanced collagen fibrosis (CML.MS/MF/AMF) were assumed to present more advanced stages mostly preceded by a megakaryocyte-rich subtype (Buhr et al., 1992). Increase in immaturity or initial transformation into an unstable phase was termed excess of blasts (EB) and manifest blastic crisis (BC) (Table 6). Although from the concept and practical point of view this classification seems to be a promising answer to the problem of histopathological staging, it should not be overlooked that this system contains a number of overlapping categories and its usefulness still needs to be tested in properly designed clinical trials. For this reason, the Hannover Classification calls for slight modification by a systematically conducted study focused on a clear-cut identification of all cell lineages and the option to correlate these features with associated laboratory data. In pursuit of this aim a corresponding clinicopathological study on more than 500 patients based on immunohistochemical and morphometric analysis and evaluation of clinical parameters resulted in a more simplified classification system. The significant correlations between bone marrow features and laboratory findings of the Cologne Classification have been assigned to a decision tree-like construction with the platelet count as the root (Table 7). This approach enables a discrimination of only three categories showing different survival patterns (Thiele et al., 1999).

In conclusion, heterogeneity of bone marrow features in CML requires a careful morphological evaluation involving trephine biopsies and appropriate means, in order to achieve a close correlation with clinical data and thus a more elaborate analysis of the disease process and to improve the impact of predictive property.

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Erratum

Volume 14, Number 4, 1999, in the article "Bone marrow histopathology in chronic myelogenous leukemia (CML)- evaluation of distinctive features with clinical impact", by J. Thiele, H.M. Kvasnicka and R. Fischer, pp 1241-1256.

Table 7 should have been included

Table 7. Cologne classification of histological subtypes in CML and correlation with clinical features.

Subtype	granulocytic	megakaryocytic	myelofibrotic
Median survival (observed)	50 months	42 months	37 months
Relevance of laboratory findings	Myeloblasts < 2% Spleen size < 3 cm	Platelet count > 500 × 10^9/l	Myeloblasts > 1% Spleen size > 2 cm
	≤ 300 × 10^9/l	Platelet count	> 300 × 10^9/l