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Megakaryocytopoiesis in haematological disorders: diagnostic features of bone marrow biopsies*

An overview

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Introduction

An increasing amount of data has accumulated from a large number of laboratories on the regulation of megakaryocytopoiesis and platelet production (Williams and Levine 1982; Gewirtz 1986; Hoffman et al. 1987; Mazur 1987; McDonald 1989; Hoffman 1989; Sims and Gewirtz 1989). A substantial body of information exists which indicates that a wide variety of alterations in development and function of this cell line plays an integral role in the pathomechanisms of thrombocytopenic or thrombocytotic disorders in man and in the evolution of chronic myeloproliferative diseases (CMPDs). A survey of the possibilities of diagnosing megakaryocytic features in routinely processed bone marrow biopsies has firstly to consider commonly used staining methods, secondly to explore readily applicable immunohistochemical reactions and finally to study the value of morphometric evaluations in the further substantiation of these lesions.

Normal megakaryocytopoiesis: histological and morphometric features

Although mature megakaryocytes are easily identifiable in bone marrow by their large size and lobulated nuclei, few groups have performed quantitative studies on these cells in man in trephine biopsies. In plastic embedded specimens (semithin sections of 3 μm thickness with Giemsa staining) values per square millimetre range between 8 ± 4 (Frisch et al. 1982, 1985), 7.59 ± 0.7 (Burkhardt et al. 1982), 7–9 (Burkhardt 1988) and 12.9 ± 2.5 (Thiele et al. 1983). Slightly higher measurements were obtained after paraffin embedding on sections with an approximate thickness of 5 μm stained by the periodic acid-Schiff reaction (PAS). In these studies the frequency of megakaryocytes per square millimetre of bone marrow ranges between 13.5 ± 2.9 and 15.3 ± 3.1 (Thiele et al. 1988a, b, 1989). It should be kept in mind that the histological features of this cell line are generated firstly by stage of maturation and polyploidy and by the exposed profiles of the different planes of sections running through the megakaryocyte (Harker 1968). Secondly, occurrence of degenerative forms (naked – denuded or pyknotic nuclei) as the outcome of completed thrombocytogenesis is an important histological aspect of megakaryocytopoiesis. Moreover, the phenomenon of emperipolesis, i.e. engulfment of haematopoietic cells into dilated cavities of the demarcation membrane system (Larsen 1970; Halil and Barrett 1980; Breton-Gorius 1981; Parmley et al. 1982; Thiele et al. 1984a; Burkhardt et al. 1984a), may have an impact on the appearance of the megakaryocytic lineage. Finally, anuclear cytoplasmic fragments and mitotic figures have to be taken into account. The striking variety in the frequency of megakaryocytes in the normal human bone marrow (range 7–15 per square millimetre), as mentioned above, may be explained by both the different methods of tissue processing and the attention paid to some or all of these elements. For this reason a refined histological evaluation and quantitation of megakaryocytopoiesis should include classification according to cell sizes (Figs. 1a–c, g, 3a–d), occurrence of mitotic figures (Figs. 1d, e, 3c, f, 4a), emperipolesis (Fig. 1f, g), naked nuclei (Figs. 1g, 4a, c), anuclear cytoplasmic fragments (Figs. 1f, 3c, e) and finally the steps of maturation (pro- and megakaryoblasts, Fig. 1i, j) as shown in Table 2. In this context, assessment of the various histological aspects must also consider planimetric variables, like circular deviation of cells and their nuclei (form perimeter) and the nuclear cytoplasmic ratio (Thiele et al. 1983, 1984b, 1988a, b, 1989, 1990a).

In contrast to the commonly used smears of marrow aspirates, the advantage of trephine biopsies is the possibility to determine the exact number of megakaryocytes together with the histotopography of this cell line within the marrow. Even more important their relationship

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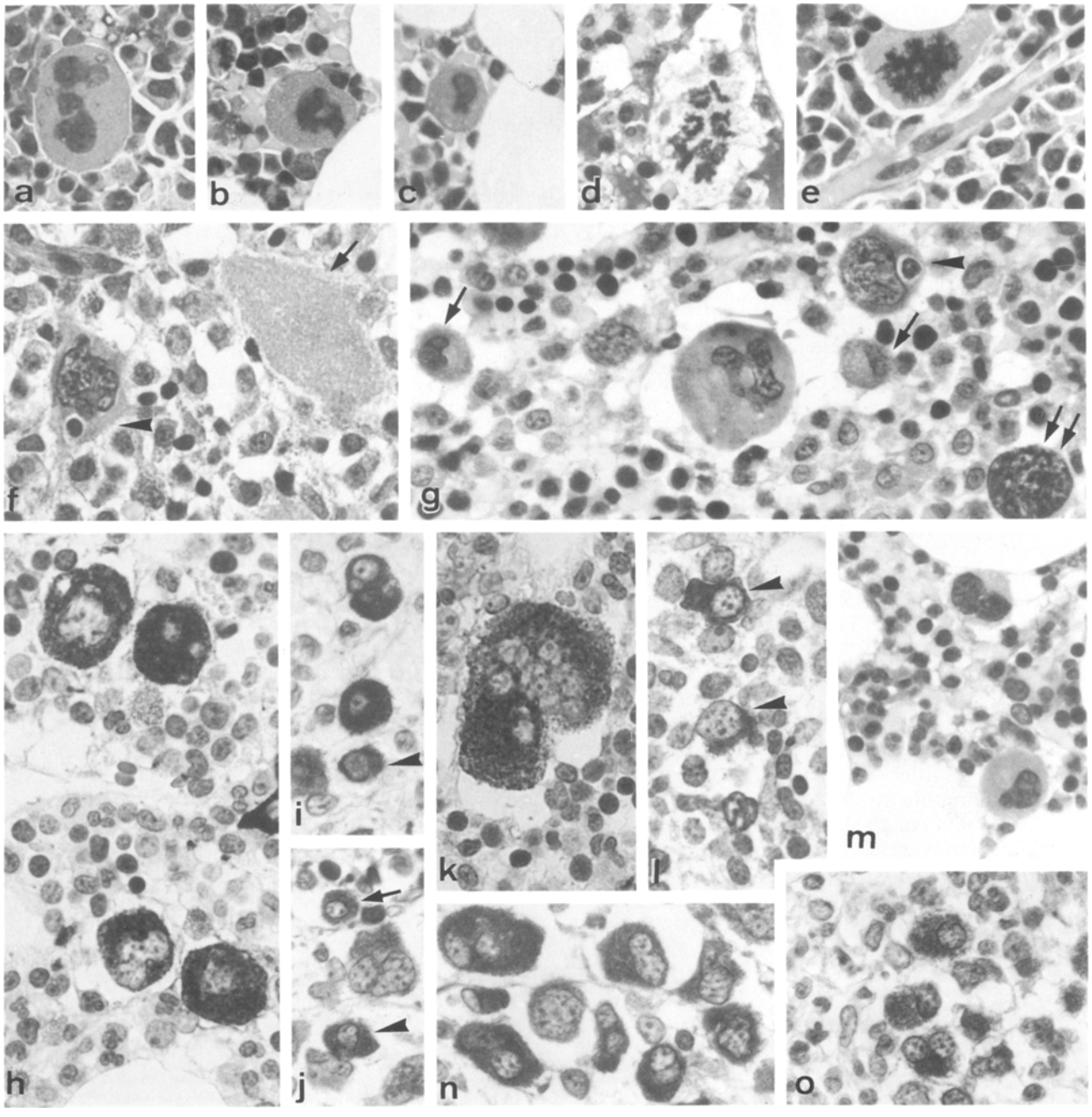


Fig. 1a–o. Classification of megakaryocyte features **a–c** Megakaryocytes in the normal bone marrow showing large (**a**), intermediate (**b**) and small sizes (**c**). **d**, **e** Different aspects of (endo)-mitosis in polyploid megakaryocytes in primary thrombocythaemia (PTH) revealing either several disparate clusters of chromatids (**d**) or a coil of chromatids with a persinusoidal localization (**e**). **f** Emperipolesis, probably of a normoblast (*arrowhead*) and large anuclear cytoplasmic fragment (*arrow*) in primary osteomyelofibrosis (OMF). **g** Small (micro-) megakaryocytes (*arrows*) in comparison with a large megakaryocyte, an emperipolesis (*arrowhead*) and a large pyknotic (bare) nucleus (*double arrow*) in polycythaemia vera rubra (P. vera). **h–l** and **n**, **o** Megakaryocytic elements following immunostaining with anti-glycoprotein IIIa (Y2/51-CD61). In comparison with mature megakaryocytes of intermediate to large sizes in reactive thrombocytosis (TH) (**h**), in chronic myeloid leukaemia (CML) (**i**) and idiopathic thrombocytopenia (ITP) (**j**) megakaryoblasts (*arrowheads*) are identifiable. A promegakaryoblast (*arrow*) with a lymphoid appearance may be also observed (**j**). Contrasting a giant megakaryocyte in PTH (**k**), megakaryocytes in myelodysplastic syndromes (MDS) (**l**) display immaturity and distortion of the nuclear-cytoplasmic ratio (*arrowheads*) which is not so conspicuous following PAS staining (**m**). In CML (**n**) a variety of intermediate to small megakaryocytes is detectable, whereas in ITP (**o**) a predominance of immature and small elements of this cell lineage may be noticed. **a–g** and **m** PAS reaction; **h–e** and **n**, **o** immunostaining with Y2/51 (CD61); **a–o** × 550

with the haematopoietic microenvironment, the fibres (Castro-Malaspina 1984; MacCarthy 1985) and vessels (Lichtman et al. 1978; Tavassoli and Aoki 1989) is evaluable. In the normal human bone marrow megakaryocytes are dispersed in the central and paracentral areas conspicuously (Burkhardt 1988) and do not reveal any tendency to touch each other (Fig. 3a). Furthermore, they display a close affinity to sinus walls but without

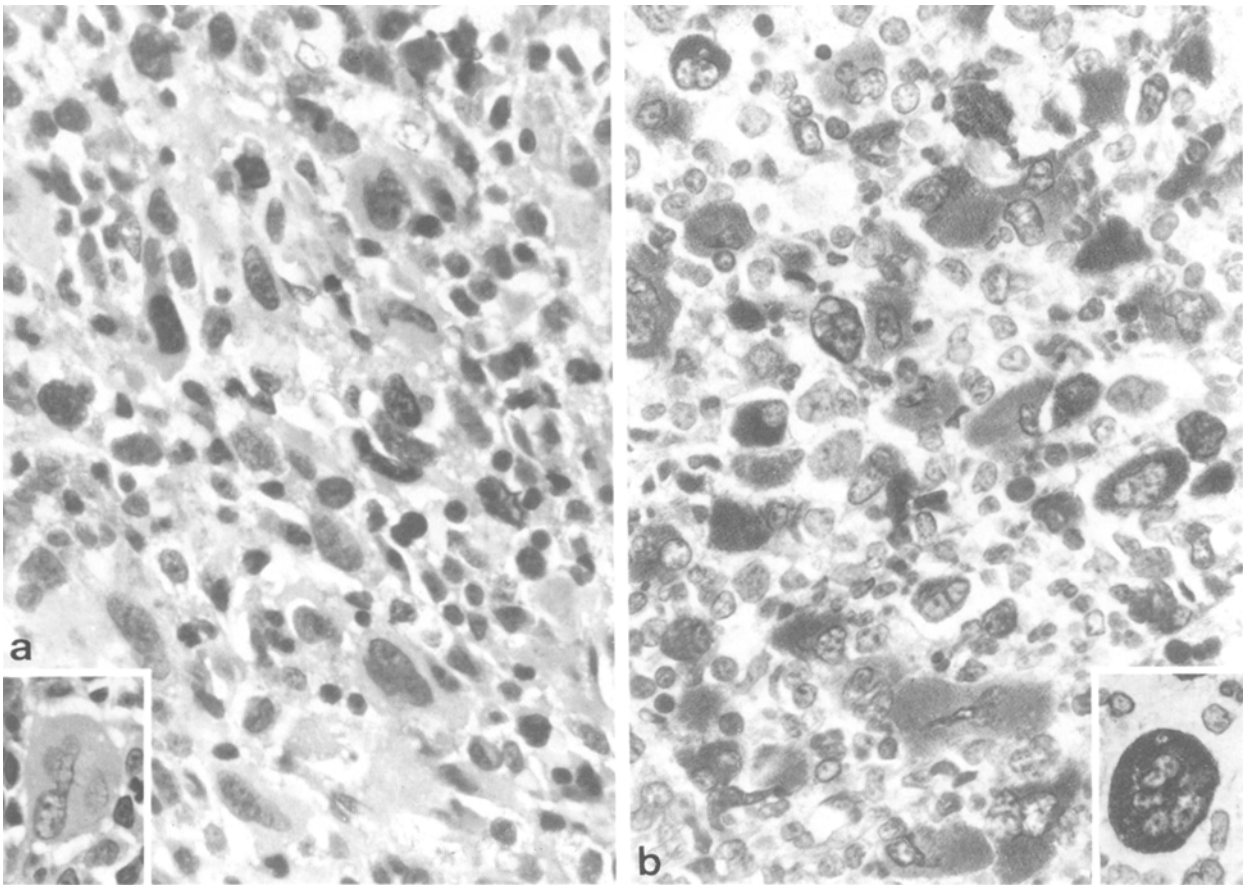


Fig. 2a, b. Immature (blastic) megakaryocytopoiesis. Acute megakaryoblastic transformation in CML with a bizarre, apparently immature cell population following PAS reaction (**a**). This cell population may be identified as of megakaryocytic origin after employment of Y2/51 (CD61) immunostaining. For comparison of normal maturation, *insets* show large megakaryocytes. **a** PAS reaction, **b** immunostaining with Y2/51 (CD61); **a**, **b** and *insets*, ×550

evidence of clustering or endosteal (peritrabecular) localization (Lichtman et al. 1978; Bartl et al. 1982; Burkhardt 1988).

Usefulness of immunohistochemistry

Megakaryocyte precursors

Initially megakaryocytopoiesis proceeds through a phase characterized by mitotic divisons of committed progenitor cells (Gewirtz 1986; Mazur 1987; McDonald 1989; Hoffman 1989). Further maturation is generally assumed to present as a continuous process of endomitotic reduplication (polyploidization) which can be divided into certain recognizable steps (promegakaryoblast – megakaryoblast / stage I – promegakaryocyte / stage II – granular megakaryocyte / stage III – platelet producing megakaryocyte / stage IV – naked (bare, pyknotic) nuclei) using standard cytological and immunocytological criteria (Williams and Levine 1982; Levine et al. 1982; Breton-Gorius and Vainchenker 1986a; Kanz et al. 1987; Mazur 1987). However, one of the most disturbing barriers to a more elaborate evaluation of the megakaryocyte series in routinely processed bone marrow biopsies has been the impossibility of applying im-

Table 1. Frequency and size of nucleated megakaryocytes in bone marrow specimens derived from various haematological disorders

	Frequency (per square millimetre)		Size (μm^2)	
	PAS	Y2/51	PAS	Y2/51
Normal bone marrow	11±3	25±5	338±48	277±175
TH	27±3	63±27	377±175	334±240
ITP	25±13	44±20	321±159	192±150
MDS	30±11	69±40	297±158	222±178
CML	37±31	75±68	254±200	190±121
P. vera	34±7	103±24	423±62	394±303
PTH	48±9	127±36	566±85	414±325
OMF	35±18	80±30	366±131	349±309

Both staining methods, the periodic acid-Schiff (PAS) reaction and immunostaining with a monoclonal antibody against glycoprotein IIIa (Y2/51-CD61) show significant differences of values. The high standard deviation of cell sizes following Y2/51 staining suggests a striking variety, including a considerable number of (small) precursor cells, not otherwise identifiable by conventional methods, like PAS. TH, Reactive thrombocytosis; ITP, idiopathic thrombocytopenia; MDS, myelodysplastic syndromes; CML, chronic myeloid leukaemia; P. vera, polycythaemia vera rubra; PTH, primary (essential) thrombocythaemia; OMF, so-called primary osteomyelofibrosis (agnogenic myeloid metaplasia)

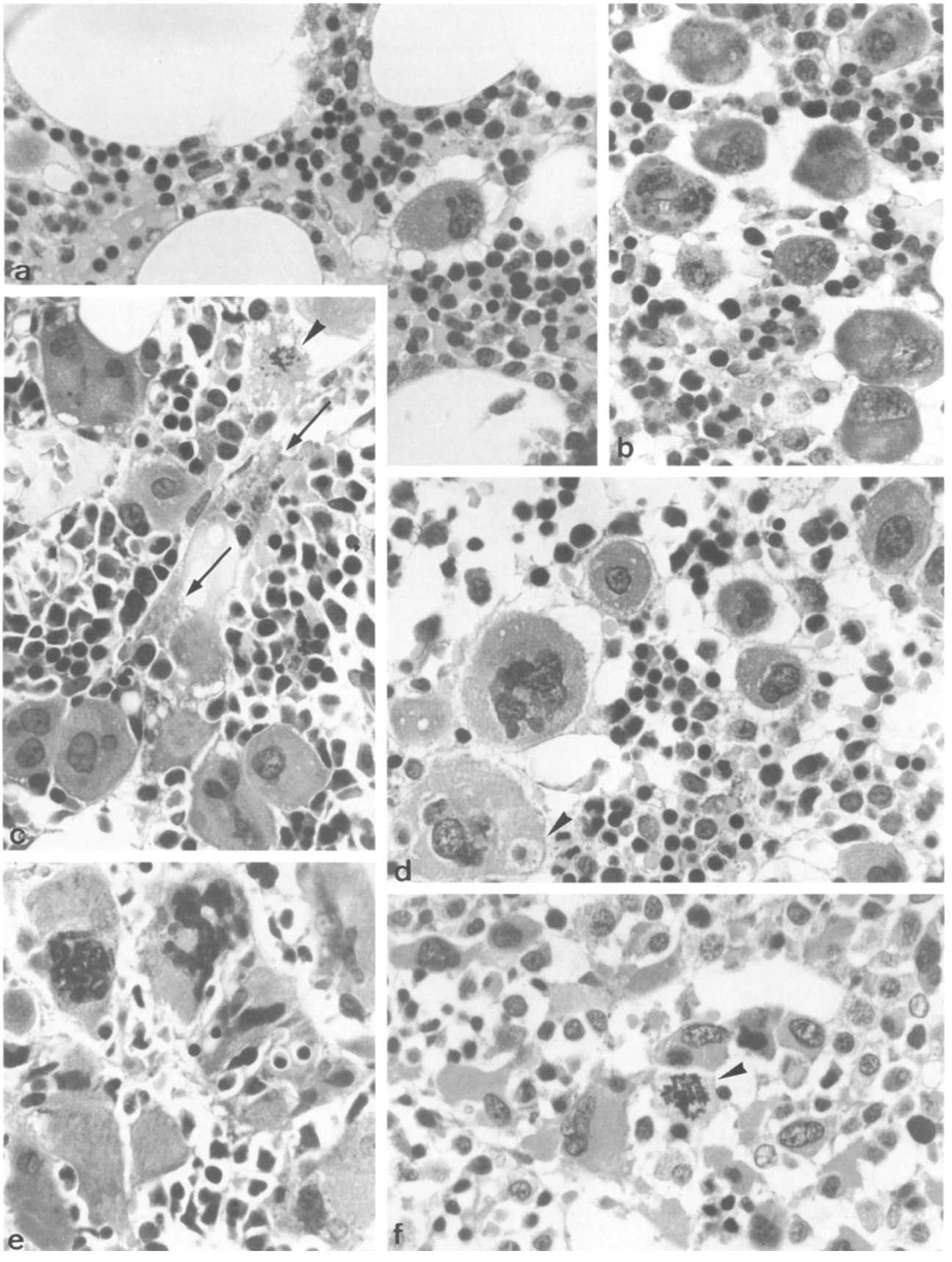
Table 2. Schematic survey of the frequency of certain histological features of megakaryocytes in normal bone marrow (N) and various disorders

	N	TH	ITP	MDS	CML	P. vera	PTH	OMF
Size classes								
Intermediate	+++	++	+	+	+	+	+	+
Giant	−	+	(+)	−	−	++	+++	+++
Micro-forms	(+)	(+)	++	+++	+++	++	−	++
Differentiation								
Immature (promegakaryoblasts)	(+)	++	++*	+*	++	++	+++	(+)
Naked (pyknotic) nuclei	(+)	++	+	++	+	++	++	+++
Cytoplasmic fragments	(+)	+	+	++	++	+	+	+++
Emperipolesis	(+)	+++	−	−	+	+++	+++	++
Mitosis	−	(+)	+	+	(+)	+	++	++
Appearance								
Pleomorphism	−	−	(+)	+	+	+++	(+)	+++
Atypia	−	−	−	++	++	(+)	−	+++
Localization								
Disseminated	+++	+++	++	++	+	+	++	−
Clustered	−	−	(+)	+	++	++	++	+++
Intravascular	−	−	−	−	(+)	+	+	+++
Peritrabecular (endosteal)	−	−	(+)	++	+	+	++	+++

Semiquantitative scoring system: (+), minimal; +, slight; ++, moderate; +++, conspicuous; −, not present; promegakaryoblasts; * disproportionate, ⁺ orderly increase; for other abbreviations, see Table 1

munohistochemical markers on formalin-fixed and paraffin-embedded material, notably monoclonal antibodies against certain platelet glycoproteins. Recently, considerable progress has been made in this field by the introduction of a monoclonal antibody against glycoprotein (Gp) IIIa, termed Y2/51-CD61 (Erber et al. 1987; Gatter et al. 1988). As a result of experimental studies, mostly on cell culture material, it is well established that monoclonal antibodies against Gp IIIa identify megakaryocyte precursors (pro- and megakaryoblasts) in addition to the mature elements of this cell line (Rabellino et al. 1981; Mazur et al. 1981; Vainchenker et al. 1982; Vinci et al. 1984; Levene et al. 1985; Ishibashi et al. 1986; Breton-Gorius and Vainchenker 1986a; Renner et al. 1987; Kanz et al. 1987; Levene et al. 1987). The combination of Y2/51 (CD61) staining reaction and morphometry yields an unusual opportunity to determine the number of megakaryocytic precursor cells in bone marrow sections or in situ and to assess the various aspects of this cell lineage in pathological conditions (Fig. 1h–l, n, o). Although it seems to be important to keep methodological pitfalls in mind (Breton-Gorius et al. 1989), the growing body of information now available is consistent with the assumption that by applying Gp IIIa antibodies, megakaryocytopoiesis may be easily characterized in routinely processed bone marrow biopsies (Erber et al. 1987; Gatter et al. 1988; Thiele et al. 1990b, c; Fox et al. 1990). Using this approach it is possible to avoid procedures such as isolation, suspension, enrichment and culture of megakaryocytic cell fractions. These may be subject to artefacts and therefore may prevent an unequivocal interpretation of results. Furthermore, by utilizing monoclonal antibodies against certain platelet glycoproteins, diagnosis of acute megakaryoblastic leukaemia – FAB M7 – has been facilitated (Tabilio et al. 1984; Koike 1984; Huang et al. 1984; Bennett et al. 1985; Velez-Garcia et al. 1985; Polli et al. 1985; Breton-Gorius and Vainchenker 1986b; Ruiz-Argüelles et al. 1986; De Oliveira et al. 1987; San Miguel et al. 1988). Similar results were obtained in acute (micro) – megakaryoblastic transformation of chronic myeloid leukaemia (CML) and other subtypes of CMPDs (Bain et al. 1977; Breton-Gorius et al. 1984; Jacobs et al. 1984; San Miguel et al. 1985; Travis et al. 1987). However, in these cases procedures for character-

Fig. 3a–f. Megakaryocytes in reactive lesions and chronic myeloproliferative disorders. **a** Normal bone marrow with dispersed appearance of a large megakaryocyte. **b** TH revealing a diffuse growth of mature, intermediate-sized to large megakaryocytes. **c** PTH with a uniform aspect of mostly large megakaryocytes, obviously of similar stages of maturation (polyploidization) deployed in and along a dilated sinus with intraluminal platelet shedding (*arrows*), two anuclear cytoplasmic fragments and further an (endo-) mitosis (*arrowhead*). **d** P. vera shows pleomorphism of megakaryocytopoiesis with an admixture of large to giant (*left half*) together with micro- and intermediate cell forms (*right half*). A giant megakaryocyte exhibits a partially exposed emperipolesis (*arrowhead*). **e** OMF with a clustering of strikingly abnormal and giant megakaryocytes in addition to anuclear cytoplasmic fragments. **f** CML of a megakaryocyte-rich subtype discloses a grouping of small megakaryocytes surrounding an (endo-) mitosis (*arrowhead*). **a–f** PAS reaction; × 550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izing megakaryoblasts were limited to smears of aspirates, frozen sections or cell suspensions and to electron microscopy. Following Y2/51 (CD61) staining identification becomes feasible on bone marrow biopsies even taken from file material or stored paraffin blocks (Fig. 2a, b), as has been shown for the megakaryoblastic crisis in CML (Thiele et al. 1990c).

Quantitation by conventional staining versus Y2/51 (anti – Gp IIIa antibody, CD61) reactivity

A comparison of morphometric data derived from standard staining methods, like haematoxylin-eosin (H&E) (Lundin et al. 1972; Kutti et al. 1973; Branehög et al. 1975), Giemsa (Burkhardt et al. 1982; Frisch et al. 1982; Thiele et al. 1982, 1983, 1984b) and PAS (Thiele et al. 1988a, b, 1989, 1990a) with Gp IIIa (Y2/51-CD61) reactivity is biased by certain differences in measurements. Following immunostaining, naked (pyknotic) nuclei are negative by definition. Moreover, anuclear cytoplasmic fragments (Table 2) have to be excluded from our calculations as they may be mistaken for giant thrombocytes, particularly in CMPDs. For this reason counts using the above-mentioned conventional staining methods must be reduced by the fraction of these two variables (only the nucleated, non-degenerative elements have to be taken into account). This assessment yields slightly lower values than those calculated without the restrictions indicated above, as may be seen for the normal bone marrow (Table 1). The rationale of this comparison is to demonstrate that the employment of easily applicable monoclonal antibodies to bone marrow sections allows a meticulous evaluation of all elements of the megakaryocytic lineage (Fig. 1h–l, n, o). In conclusion, a significant increase in the number of Y2/51-positive megakaryocytes in the normal bone marrow as well as in a variety of haematological disorders (Table 1) implies that a considerable amount of precursors (pro- and megakaryoblasts) are included (Fig. 1i, j). These early megakaryocytic elements stain in addition to the mature forms (promegakaryocytes to mature megakaryocytes stage IV) or micromegakaryocytes which may be identified by the PAS reaction (Fig. 1a–c, f, g, m). In congruence with this assumption, the mean values of cell sizes are greatly reduced due to the large numbers of the smaller immature subpopulation.

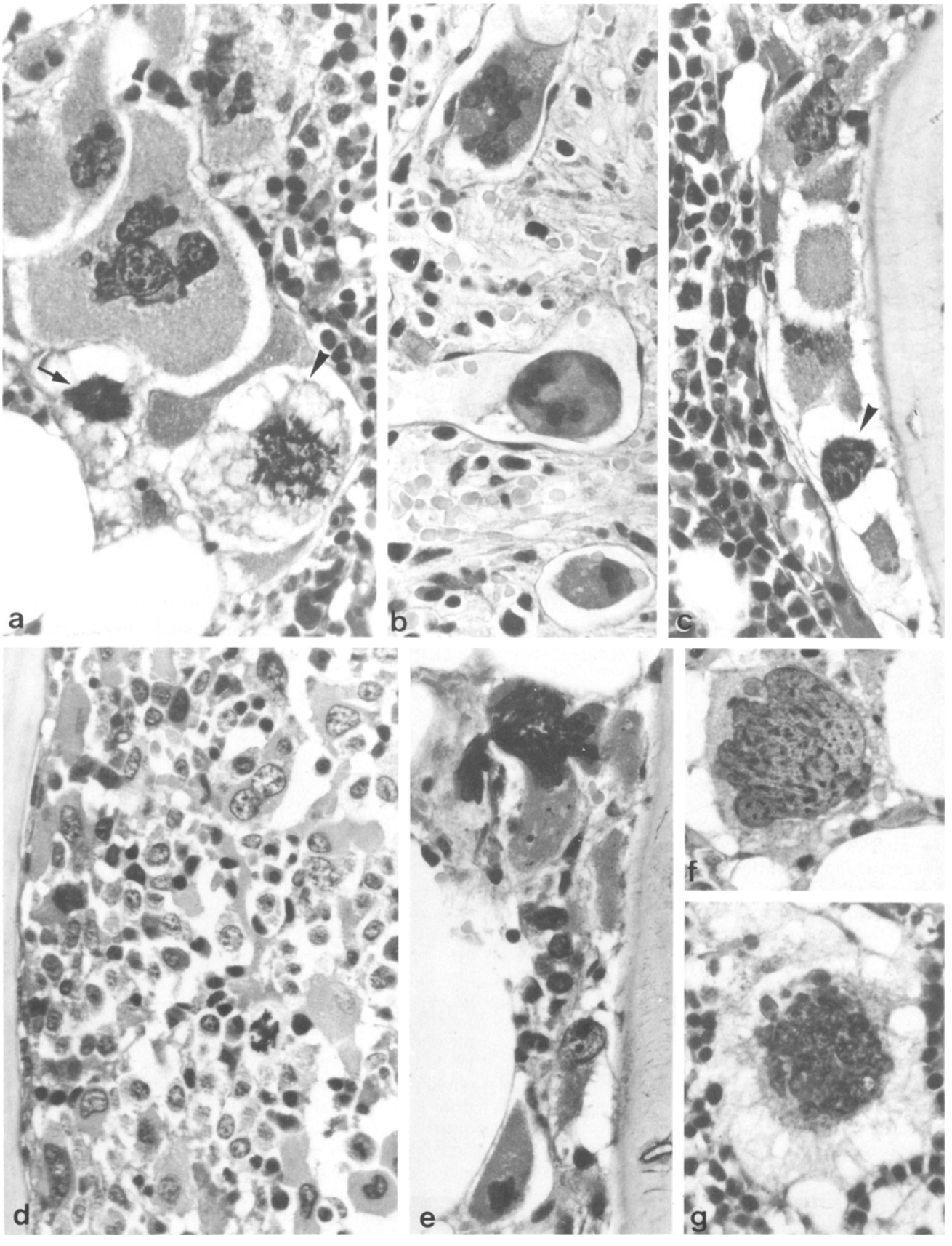
Histological criteria for classification of megakaryocytic lesions

As mentioned above, megakaryocytes reveal a remarkably dispersed pattern of growth in the normal bone marrow neighbouring the sinus walls of the central and paracentral (intermediate) areas (Fig. 3a). In addition to immaturity (Fig. 1i, j, l, n, o), increase in this cell line causes either a diffuse (Figs. 1h, 3b–d) or clustered appearance (Figs. 3e, f, 4a). These changes may further result in a dislocation towards the endosteal (paratrabecular) generation zone of granulocytopoiesis (Fig. 4c–e). Additionally, all these features may be associated with occurrence of abnormal sizes (micromegakaryocytes, Figs. 1g, m, 3f, 4d) and large to giant forms (Figs. 3e, 4a, e) arranged in an uniform (Fig. 3b, c) as well as pleomorphic pattern (Figs. 1g, 3d). Moreover, a pronounced intravascular growth of megakaryocytes (Fig. 4b, c) including various stages of maturation as well as degenerative changes may be detectable (Fig. 4c). In comparison with reactive lesions (Figs. 1h, 3b), gross anomalies are exhibited not so much by the frequent presence of mitotic figures (Figs. 1d, e, 3c, f, 4a) or an interstitial (Fig. 4g) instead of an intrasinusoidal (Fig. 3c) platelet shedding. Abnormalities are expressed most prominently by an apparent disorganization of nuclear-cytoplasmic development (Figs. 3e, f, 4a, d–f). These atypias generate bizarre appearances of megakaryocytopoiesis in combination with the abnormal topographical distribution, that is to say clustering and paratrabecular dislocation (Figs. 3e, f, 4a, d, e).

Reactive and pre-neoplastic disorders

Reactive thrombocytosis (TH) is not an unusual finding in a variety of conditions of inflammatory origin (chronic rheumatoid arthritis, Crohn's disease, hepatitis), in metastatic carcinoma, iron deficiency anaemia and following splenectomy (Murphy 1983; Burkhardt 1988). An increased number of megakaryocytes of mostly intermediate to larger size and a diffuse growth pattern (Table 1; Figs. 1h, 3b) is observable in bone marrow sections with the frequent occurrence of emperipolesis (Table 2). There is no obvious disturbance of nuclear-cytoplasmic maturation and the number of precursor elements reveals a proportionate expansion. This appearance of the megakaryocytic lineage in TH differs from features recognizable in *idiopathic (immune-) thrombocytopenia (ITP)*. Although there is also a high number of megakaryocytes (Harker 1970; Branehög et al. 1975;

Fig. 4a–g. Abnormalities of megakaryocytopoiesis (histotopography and differentiation). **a** Cluster of bizarre megakaryocytes including a giant form and a large (endo-) mitosis (*arrowhead*) in addition to a pyknotic (denuded) nucleus (*arrow*) in OMF. **b, c** Conspicuous intravascular growth of either large megakaryocytes (**b**) or a variety of megakaryocytic elements ranging from pyknotic – degenerative forms (*arrowhead*) to anuclear cytoplasmic fragments in an endosteal vessel (**c**) in OMF. **d, e** Abnormal localization of megakaryocytes in a megakaryocyte-rich subtype of CML with a sheet-like proliferation of atypical micromegakaryocytes along the endosteal border of the spongious trabeculae with extention towards the paracentral (intermediate) marrow space (**d**). A similar dislocation of large and bizarre looking megakaryocytes may be found in OMF (**e**). **f, g** Giant megakaryocytes in OMF frequently display signs of immaturity with a dispersed pattern of nuclear chromatin and only a small portion of cytoplasm (**f**). Occasionally, they exhibit an interstitial shedding of platelets with a veil-like appearance surrounding a dense multi-lobulated nucleus (**g**). **a–g** PAS reaction; ×550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Brannan and Guthrie 1988) as in TH (Table 1), smaller sizes are predominant which suggests a considerable subpopulation of precursor forms (Table 1; Fig. 1j, o). This assumption of an increased number of pro- and megakaryoblasts in ITP (Table 2; Fig. 1j) confirms in vitro results on cultured progenitor cells of the bone marrow in this condition (De Alarcon et al. 1987). However, in comparison with the normal bone marrow, megakaryocyte sizes in ITP reveal a remarkably high standard deviation of values implying an admixture of small immature and larger cell forms (Table 1). This latter finding is an agreement with results gained from bone marrow aspirates, which revealed larger sizes (Branehög et al. 1975) and correspondingly higher ploidy classes in some cases, when compared with control specimens (Knecht and Streuli 1985; Mazur et al. 1988). It seems that the common morphological changes observed in ITP, like elevation of megakaryocyte number and a predominance of small precursors, are related to the peripheral platelet count and reflect enforced platelet production (Harker 1970; Queisser et al. 1971; Branehög et al. 1975; Eldor et al. 1989). Measurements of thrombocyte production in this disorder have disclosed that megakaryocytopoiesis and platelet turnover rate are increased in parallel, to as much as eight times normal (Harker 1970).

Myelodysplastic syndromes (MDS) are generally diagnosed and classified by examination of bone marrow smears and not primarily by evaluation of biopsy material (Bennett et al. 1982; Jacobs 1985). However, dysmegakaryopoiesis, the presence of a significantly increased amount of abnormal (micro-) megakaryocytes (Table 1, Fig. 1l, m) with a striking variability in appearance, including immature elements and clustering, has previously been described as a conspicuous feature in this disorder (Thiele et al. 1980; Fohlmeister et al. 1985a, b; Frisch et al. 1985; Burkhardt 1988; Frisch and Bartl 1986; Georgii et al. 1987). These abnormalities of megakaryocytopoiesis are readily recognizable from bone marrow biopsies following immunostaining with Y2/51 (Fig. 1l) as has most recently been shown by Fox et al. (1990). The number of megakaryocytes could not be related to cellularity or to hypo- and hyperplastic subtypes of MDS (Tricot et al. 1984; Fohlmeister et al. 1985a, b; Delacretaz et al. 1987; Yoshida et al. 1988; Kitagawa et al. 1989). In addition to the frequent appearance of pleomorphic micromegakaryocytes (Thiele et al. 1980; Wisneth et al. 1980; Fohlmeister et al. 1985a, b; Frisch et al. 1985), as a further anomaly a topographical disturbance with immature elements located along the trabecular surfaces has been emphasized (Frisch et al. 1985; Frisch and Bartl 1986; Fox et al. 1990). Functional defects of megakaryocytopoiesis in MDS were documented by an abnormal colony formation of progenitor cells from the bone marrow (Juvonen et al. 1986). Disarray of megakaryocyte development was also suggested by a disproportionate increase in precursors, like pro- and megakaryoblasts, as well as by strikingly high values for the nuclear-cytoplasmic ratio (Fig. 1l, m). An overview of these disease features is given in Table 2 as a semiquantitative evaluation in comparison with the normal marrow and the different subtypes of CMPDs.

Chronic myeloproliferative diseases

The main subtypes of CMPDs are CML, *polycythaemia vera rubra* (P. vera), *primary (essential) thrombocythaemia* (PTH) and so-called *primary (idiopathic) osteomyelofibrosis-sclerosis* or *agnogenic myeloid metaplasia* (OMF). In these disorders, variety in megakaryocyte numbers, sizes and other histopathological features, accompanied by different aspects of granulo- and erythrocytopoiesis have been described repeatedly (Ellis et al. 1975; Ellis and Peterson 1979; Georgii et al. 1984a, b; Burkhardt et al. 1984b; Frisch et al. 1984, 1985; Burkhardt 1988; Frisch and Bartels 1985; Wolf and Neiman 1985; Lorand-Metze et al. 1987). These findings were confirmed and further substantiated by morphometric measurements (Lundin et al. 1972; Kutti et al. 1973; Branehög et al. 1975; Thiele et al. 1982, 1983, 1984b, 1990b; Lazzarino et al. 1986). In particular in CML, a remarkable range in the counts for megakaryocytes was evident (Table 1) and could be correlated with the evolution of myelofibrosis and with prognosis (Burkhardt et al. 1984b; Georgii et al. 1984b, 1990; Lazzarino et al. 1986; Burkhardt 1988; Lorand-Metze 1989; Thiele et al. 1990a). A recently performed study by Rozman et al. (1989) however, was unable to establish relevant clinical or prognostic differences between so-called granulocytic and megakaryocytic subtypes of CML (Burkhardt et al. 1984b, 1986, 1990; Burkhardt 1988; Frisch et al. 1984, 1985; Georgii et al. 1984a, b, 1990). In CML there is a conspicuously wide range in the counts for megakaryocytes (Thiele et al. 1983, 1990b) as demonstrated by the high standard deviation of values shown in Table 1 and in addition, a predominance of atypical smaller cell forms (micromegakaryocytes) with hypolobulation of their nuclei (Figs. 1i, n; 3f, 4d). In P. vera a significant increase in the number of megakaryocytes is encountered when compared with the other CMPDs (Table 1). However, these reveal very disparate sizes, which range from micromegakaryocytes to giant elements and thus generate a pleomorphic appearence in the cell line (Figs. 1g, 3d). These features present a strikingly different aspect, distinguishable from PTH (Thiele et al. 1988b) where large or giant megakaryocytes predominate with no apparent anomaly of maturation (Fig. 3c). Discrimination of P. vera from PTH is feasible by bone marrow histopathology (Thiele et al. 1988a, b) although this has been rejected by findings obviously derived from marrow aspirates (Iland et al. 1983, 1987; Murphy 1983; Murphy et al. 1986). Morphology of megakaryocytes in OMF differ significantly from those of P. vera and PTH by showing a grossly disturbed organization with frequent occurrence of bizarre elements (Figs. 3e, 4a–c, e). Following morphometric evaluation, it is evident that the increase in medullary fibrosis, characteristic of OMF was paralleled by an increasing pleomorphism of megakaryocytes. This feature included irregularities of shape factors and frequency and size of pyknotic (bare) nuclei particularly (Thiele et al. 1989). In a semiquantitative evaluation Table 2 summarizes diagnostic features of megakaryocytes in the different subgroups of CMPDs.

To correlate histomorphological findings with clinical and laboratory data, but particularly with prognosis, attempts were made to classify CMPDs with special emphasis on megakaryocytopoiesis (Burkhardt et al. 1984b, 1986, 1990; Georgii et al. 1990). Following the histological features described in Table 2, a number of striking differences between the main clinical entities are demonstrable; however, overlaps are not excluded. It is debatable whether the examination of megakaryocyte morphology alone or in combination with the other bone marrow lesions enables a clear cut diagnosis in every patient without knowledge of laboratory data and cytology. These difficulties are obvious in specimens revealing advanced myelofibrosis together with a prominent megakaryocytic proliferation or the so-called myelofibrosis-osteomyelosclerosis syndrome (Burkhardt et al. 1984b, 1986). Consequently the various features of megakaryocytopoiesis which have to be regarded as a most valuable aid for the diagnosis of haematological disorders should always be interpretated with reference to alterations of the surrounding bone marrow and corresponding clinical findings. The need for such a synoptical approach towards the problems of megakaryocyte morphology is fundamental for the classification of CMPDs.

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