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Diagnostic differentiation of essential thrombocythaemia from thrombocythaemias associated with chronic idiopathic myelofibrosis by discriminate analysis of bone marrow features - a clinicopathological study on 272 patients

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Summary. Until now diagnosis of essential thrombocythaemia (ET) is generally performed by following the criteria of the Polycythaemia Vera Study Group (PVSG) that only marginally regards morphological features. Bone marrow biopsies were studied from 272 patients with ET in strict accordance with the PVSG guidelines and also from 35 control patients with reactive thrombocytosis. To define morphological features of distinctive impact more accurately, we performed a stepwise discriminant analysis of 16 morphological parameters based on histochemical staining reactions and semiquantitative grading of standardized features. A clear-cut separation into three distinctive histological patterns was accomplished that showed in more than 96% a correct predicted classification. Variables of significant impact included fibre content, quantity and cytological abnormalities of megakaryopoiesis like bulbous (cloud-like) nuclei, degree of nuclear lobulation and presence of giant forms. These changes were not detectable in the control group. The different constellations of histopathological features could be assigned to true ET (98 patients) and false ET, i.e. 136 patients with prefibrotic and 38 patients with early fibrotic chronic idiopathic myelofibrosis (IMF) accompanied by thrombocythaemia. A re-evaluation of clinical findings was in keeping with this classification into three categories that exerted significant differences to develop myelofibrosis during observation time and also different survival patterns. Contrasting IMF true ET is characterized by a pronounced proliferation of the megakaryocyte lineage showing large to giant cells without maturation defects and no relevant increase in reticulin fibres. Discrimination between these entities is warranted, because of a significant difference in presenting haematological data, follow-up and life expectancy.

Key words: Essential thrombocythaemia, Megakaryopoiesis, Fibres, Discriminate analysis, Bone marrow biopsies

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Introduction

Definition of more complex morphological features that exert a distinctive impact on diagnosis and classification is often hampered by subjective ranges of interpretation. Considering the various classifications of malignant lymphomas and bone marrow lesions in chronic myeloproliferative disorders (MPDs), this phenomenon is of special importance in haematopathology. A promising approach to cope with this situation includes discriminate analysis based on standardized morphological features in combination with semiquantitative grading systems. The aim of this method is to recognize characteristic patterns of histopathology that allow a clear-cut distinction between different disease entities. In this context conflict and discussion still persists concerning bone marrow lesions in essential thrombocythaemia (ET) that exert a discriminating value in comparison with the other or allied subtypes of MPDs. According to the revised criteria of the Polycythaemia Vera Study Group (PSVG), diagnosis of ET is one of exclusion and does only marginally consider bone marrow morphology (Murphy et al., 1997). This shortcoming reflects the need for more sensitive laboratory tests to separate either between ET and reactive thrombocytosis (RT) or thrombocythaemias eventually associated with the various subtypes of MPDs, but especially with chronic idiopathic myelofibrosis (IMF). A number of attempts have been made to improve this adverse situation (Michiels and Juvonen, 1997). The latter include positive criteria like

cell culture studies (Juvonen et al., 1993; Florensa et al., 1995; Westwood and Pearson, 1996), characteristic clinical findings (Iland et al., 1983; Chistolini et al., 1990; Colombi et al., 1991; Van Genderen and Michiels, 1993; Jantunen et al., 1998), specific functional alterations of the platelets (Dudley et al., 1989) and in particular histopathology of the bone marrow (Thiele et al., 1988, 2000; Georgii et al., 1990, 1998; Buhr et al., 1992; Bartl et al., 1993). On the other hand, no specific cytogenetic or molecularbiological marker is presently known to distinguish ET from the other MPDs and RT either. However, diagnostic reliability may be greatly enhanced by including spontaneous megakaryocyte colony formation and specific features of histopathology into the set of relevant criteria (Michiels and Juvonen, 1997). Despite various proposals (Michiels and Juvonen, 1997; Thiele et al., 1999a, 2000), so far many clinicians are reluctant to consider these points in ongoing or prospective clinical trials. Arguments that are not in favour of modifying the diagnostic guidelines of the PVSG (Murphy et al., 1997) emphasize the fact that cell culture studies are burdensome and time-consuming and dependent on professional interpretation of results (Westwood and Pearson, 1996). Moreover, it has been stated that standardized objective criteria for bone marrow evaluation have not yet been established for a positive and independent morphological diagnosis, since the majority of previous histological evaluations were performed without explicitly regarding the PVSG criteria (Thiele et al., 1988; Georgii et al., 1990, 1998; Buhr et al., 1992; Bartl et al., 1993). Therefore in ET determination of bone marrow histopathology by means of discriminate analysis of standardized features (semiquantitative evaluation) is warranted and should be performed on a large series of patients conforming with the postulates of the PSVG (Tefferi et al., 1995; Murphy et al., 1997). For this reason, the purpose of the present study was to enhance diagnostic validity by investigating whether a characteristic pattern of histopathology may be associated with the clinical diagnosis of ET. In addition we tried to elucidate whether these histological features are reproducible and, regarding laboratory parameters, are consistent with a distinctive disease entity.

Materials and methods

Patients

This retrospective clinicopathological study was based on 272 adult patients (105 men, 167 women, median age 60 years) who presented between 1982 and 1994 findings in strict accordance with the updated criteria of the PVSG for the diagnosis of ET (Tefferi et al., 1995; Murphy et al., 1997). These included: a platelet count $\geq 600x10^9$/l determined at intervals of at least two months, a haematocrit <40% or a normal red blood cell mass; no Philadelphia chromosome; no evidence of myelodysplastic syndromes (MDS); no reactive thrombocytosis; stainable iron deposits in the bone marrow or normal red blood cell mean corpuscular volumen; and finally, no collagen fibrosis or <1/3 biopsy area without both marked splenomegaly and leuko-erythroblastic reaction. In addition 35 patients (15 men, 20 women) with reactive thrombocytosis (RT) and an elevated platelet count ranging from 480 to $850x10^9$/l due to a variety of conditions (rheumatoid arthritis, Crohn's disease, splenectomy, metastasizing cancer, iron-deficiency anaemia) served as controls.

Bone marrow biopsies

Representative pretreatment trephine biopsies of the bone marrow (length 14.2±1.9 mm) were performed from the posterior iliac crest at diagnosis and additionally in 134 of the 272 patients during observation time (mean interval 39±32 months). Fixation was carried out in an aldehyde solution for 12-48 hours (2 ml 25% glutaraldehyde, 3 ml 37% formaldehyde, 1.58 g anhydrous calcium acetate, and distilled water per 100 ml). Further processing included decalcification for 3-4 days in 10% buffered ethylene-diamine tetra-acetic acid (EDTA), pH 7.2, paraffin embedding, and employment of several routine staining techniques, involving Haematoxylin and Eosin (H&E), Giemsa, PAS (periodic acid Schiff reagent), naphthol-AS-D-chloroacetate esterase, Perls' reaction for iron and a silver impregnation method following Gomori's technique. For a more specific staining of the different haematopoietic cell lineages two monoclonal antibodies were selected: CD61 (anti-platelet glycoprotein IIIa) for the identification of megakaryocytes including precursor cells (pro-megakaryoblasts and megakaryoblasts) and Ret40f (anti-glycophorin C) to label erythropoiesis. Monoclonal antibodies and other reagents were purchased from Dako-Diagnostica GmbH (Hamburg, Germany). Details of staining procedures (APAAP-method) have been reported in previous communications (Thiele et al., 1999b, 2000).

Discriminate analysis of histological features

The histological slides were reviewed independently by two observers without prior knowledge of clinical data except for the putative clinical diagnosis of ET. The following histological parameters were considered for morphological evaluation (Table 1): 1. Overall bone marrow cellularity compared to the age-matched control (Thiele et al., 1988, 1999b; Georgii et al., 1998); 2. Quantity of erythropoiesis with regard to normal values (Thiele et al., 1999b); 3. Immaturity (left shifting) of nucleated erythroid precursors; 4. Normal or abnormal maturation of erythropoiesis; 5. Amount of neutrophil granulopoiesis (Thiele et al., 1999b); 6. Immaturity (left-shifting) of the myeloid lineage with dislocations from the bone trabeculae to the central marrow space; 7. Quantity of megakaryocytes with respect to the reported normal values (Kaloutsi et al., 1991; Thiele et al.,

1999b); 8. Clustering of megakaryocytes implying aggregates of more than three cells lying adjacent to each other; 9. Size of megakaryocytes with differentiation into dwarf (small) and giant forms according to relevant classifications (Thiele et al., 1988; Nafe et al., 1991; Bartl et al., 1993); 10. Extent of nuclear lobulation of megakaryocytes (Nafe et al., 1991); 11. Maturation defects implicating abnormalities of nuclear-cytoplasmic development (bizarre features); 12. Presence of bulbous (hyperchromatic) nuclei including at least three definitively hypolobulated (cloud-like) nuclei (Buhr et al., 1992; Georgii et al., 1998; Thiele et al., 2000); 13. Presence of denuded (naked) nuclei of megakaryopoiesis (Thiele et al., 1988; Buhr et al., 1992); 14. Increase and thickening of reticulin fibres exceeding the normal content by a three-fold amount consistent with early reticulin fibrosis (Bauermeister, 1971; Thiele et al., 1988, 1999b); and 15. Presence of lymphoid nodules (Georgii et al., 1998).

All parameters (scores) were entered into a computer database system providing intense checks for logical sequence and consistency.

Statistical analysis

The diagnostic importance of these standardized bone marrow features was tested by means of stepwise discriminant analysis. The aim of this method is to obtain a small set of relevant discriminating variables that provide a reliable prediction of group classification (Everitt and Dunn, 2001). In this context it should be explicitly mentioned that to enhance sensitivity of this calculation, primarily evaluation of the histological parameters was based on histochemical and immunohistochemical staining techniques: neutrophil granulopoiesis - naphthol-AS-D-chloroacetate reaction, erythropoiesis - Ret40f and megakaryocytes - CD61. However, following a proper scoring and assessment of these samples, in a pilot study we tried successfully to reproduce these parameters by using H&E-stained slides in particular regarding the variable features of megakaryopoiesis. To calculate statistically significant differences in the expression of clinical and haematological variables in the different histological categories we used the non-parametric Mann-Whitney U-Test. Survival rates were determined for each group and additionally, the proportion of life loss due to the underlying disease was calculated to adjust survival of our patients to general mortality by using methods detailed in previous papers (Hakama and Hakulinen, 1977; Hakulinen, 1982; Kvasnicka et al., 1997).

Table 1. Descriptive analysis of histological features (semiquantitative evaluation with reference to age-related normal values) in 272 patients presenting with presumptive ET according to the updated PVSG criteria.

PARAMETER	BREAKDOWN (NUMBER) OF PATIENTS						
	overt decrease (-3)	moderate decrease (-2)	slight decrease (-1)	normal or absent (0)	slight increase (+1)	moderate increase (+2)	overt increase (+3)
1. Cellularity	-	-	-	49	192	31	-
2. Erythropoiesis							
Quantity	-	1	70	180	20	1	-
Left shift	-	-	-	238	34	-	-
Maturation defects	-	-	-	253	18	1	-
3. Granulopoiesis							
Quantity	-	-	-	81	148	43	2
Left shift	-	-	-	178	94	-	-
4. Megakaryopoiesis							
Quantity	-	-	-	-	114	140	18
Clusters	-	-	-	55	145	69	13
Size:							
small	-	-	-	12	121	123	16
giant	-	-	-	2	107	139	24
Nuclear lobulation	5	58	104	21	46	34	4
Maturation defects	-	-	-	95	101	67	9
Bulbous nuclei	-	-	-	97	89	77	9
Naked nuclei	-	-	-	68	183	21	-
5. Myeloid stroma							
Reticulin fibres	-	-	-	233	38	1	-
Lymphoid nodules	-	-	-	208	46	14	4

Results

Contrasting the presumptive clinical diagnosis of ET histological features of the bone marrow revealed a strikingly expressed heterogeneity. Following immunohistological stainings either normal cellularity and myeloid proliferation was found or in a considerable number of biopsies an increase in neutrophil granulopoiesis was revealed (Fig. 1a,b). Nucleated erythroid precursors showed normal-sized islets, but more often a reduction (Fig. 1b,d). However, most conspicuous were alterations related to the appearance of megakaryopoiesis and fibre density. Arrangement of megakaryocytes within the marrow (histotopography) ranged from random to dense clustering (Figs. 2a,c, 3d). Cytology included large to giant mature megakaryocytes with deeply lobulated staghorn-like nuclei (Fig. 2a,c,d). On the other hand, a large number of specimens exhibited groups of abnormal giant to very small megakaryocytes displaying a significant disturbance of maturation with bulbous (hypolobulated) and hyperchromatic nuclei that generated a dysplastic or bizarre aspect (Fig. 2b,e,f). Frequently naked (denuded) nuclei of different sizes were detectable (Fig. 3a). The interstitial space also exhibited a variety of pathologies with or without a mild increase in reticulin associated with clustered megakaryocytes (Fig. 3b-d) and rarely one to three well-marginated lymphoid nodules (Fig. 3e). Following a semiquantitative scoring of the most prominent features, significantly different ratings were observed (Table 1). All these features were not expressed in the control group with RT. In these samples megakaryopoiesis revealed a random distribution throughout the marrow with small to medium-sized cells lacking cytological anomalies such as cloud-like, poorly-segmented or grossly hyperlobulated nuclei.

Following semiquantitative gradings of morphological features to obtain a small number of useful variables, a stepwise discriminant analysis was applied. The aim of this method was to provide a discriminant function that enables the distinction of new cases. By classifying the cases that were used to derive this function in the first place and by comparing predicted group memberships with actual memberships, we were able to empirically measure the success in discrimination by observing the proportion of correct classification. Thus, by tabulation of the predicted group memberships one can validate the efficacy of these discriminating variables. In a first trial we focused on samples that showed no reticulin fibrosis (Fig. 3b,c) with the intention to distinguish true ET from prefibrotic stages of IMF (IMF-0) with associated thrombocythaemia (Table 2). Further analysis included a separation into three histological patterns which proved to be clearly distinguishable from each other (Table 3). Applying the set of discriminating variables 96% of our cases were classified correctly to their underlying histological group (predicted membership). It is reasonable to assume that the significant heterogeneity of bone marrow histopathology in presumptive ET is properly reflected by the wide ranges of scorings of standardized morphological parameters (Table 1). This remarkable feature may certainly be related to an admixture with initial (prefibrotic) and early IMF (IMF-0, IMF-1). Classification of our series of 272 patients with the putative clinical diagnosis of ET (Tefferi et al., 1995; Murphy et al., 1997) revealed 98 patients with true ET and 174 patients with false ET, i.e. 136 patients with IMF-0 and 38 patients with IMF-1 according to their distinctive patterns of bone marrow morphology (Table 3). Altogether, our findings suggest that the following standardized histopathological features exert a distinctive diagnostic impact on the separation of true ET from initial and early IMF: reticulin fibres, abnormalities of the megakaryocyte lineage including bulbous (cloud-like) nuclei, extent of nuclear lobulation, presence of giant forms and quantity of megakaryocytes.

Table 2. Stepwise discriminant analysis of standardized bone marrow features (Table 1) showing distinctive patterns between ET and prefibrotic stage IMF (IMF-0), especially regarding the megakaryocyte lineage.

VARIABLE	ET	VERSUS	IMF-0
1. Megakaryocytes:			
Presence of bulbous nuclei	-		+
Increase in nuclear lobulation	+		-
Increase in quantity	-		+
2. Increased cellularity	-		+
3. Megakaryocytes:			
Presence of maturation defects	-		+
Increase in giant megakaryocytes	+		-
No. of patients	98		136

Statistical analysis: Wilks' lambda statistics = 0.210; p<0.0001. Predicted group membership: ET, 96/98 (98%); IMF-0, 130/136 (95,6%); 96.6% of original grouped cases correctly classified.

Table 3. Stepwise discriminant analysis of standardized bone marrow features (Table 1) revealing distinctive patterns between ET, prefibrotic IMF (IMF-0) and early IMF (IMF-1) including fibre content and megakaryocyte abnormalities.

VARIABLE	ET	VERSUS	IMF-0	VERSUS	IMF-1
1.Increase in reticulin fibres	-		-		+
2. Megakaryocytes:					
Presence of bulbous nuclei	-		+		+
Increase in nuclear lobulation	+		-		-
Increase in giant forms	+		-		-
Increase in quantity	-		+		+
No. of patients	98		136		38

Statistical analysis: Wilks' lambda statistics = 0.240; p<0.0001. Predicted group membership: ET, 96/98 (98%); IMF-0, 127/136 (93.4%); IMF-1, 38/38 (100%); 96.6% of original grouped cases correctly classified.

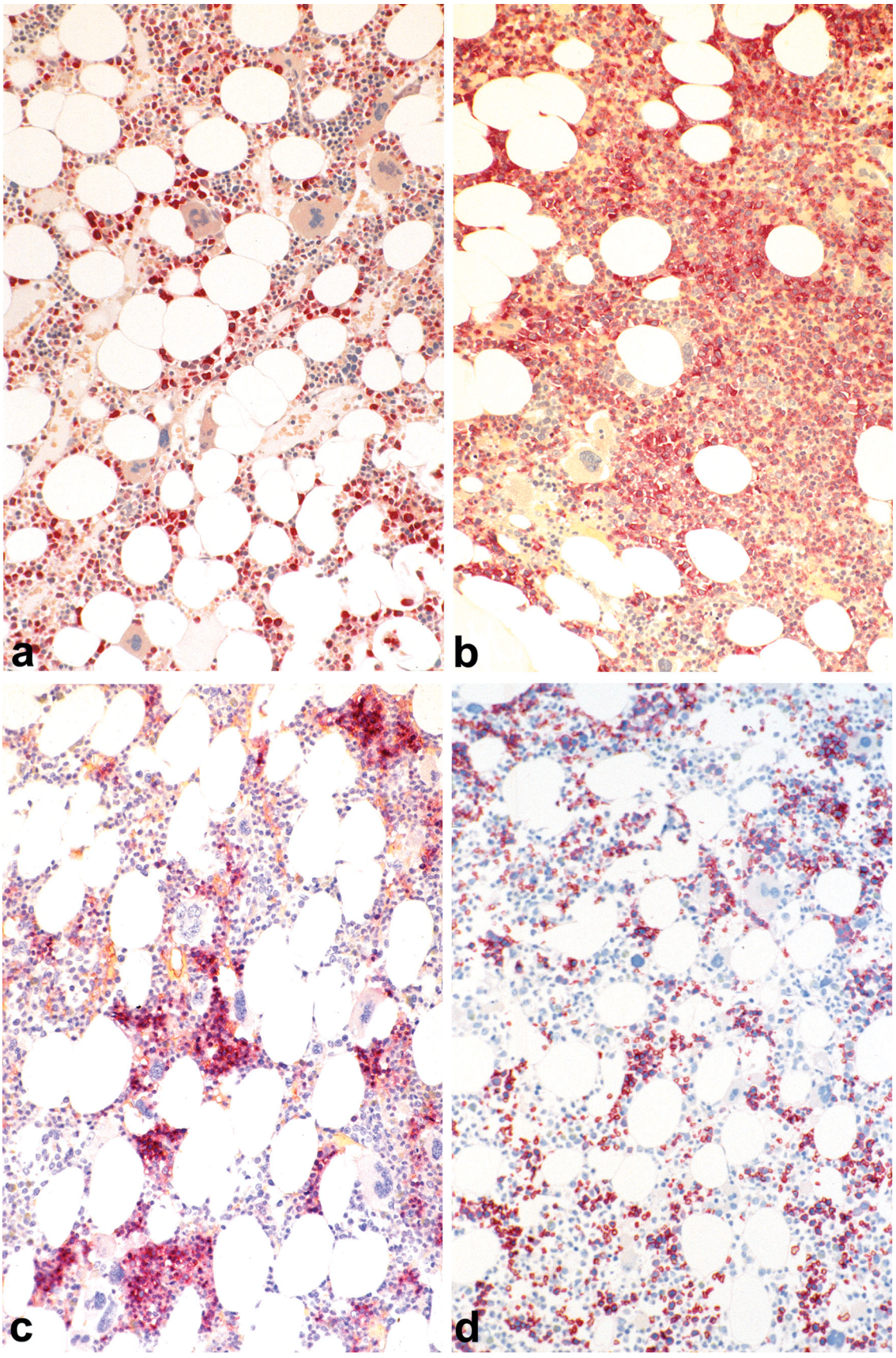

Fig. 1. Cellularity, granulo- and erythropoiesis. Normal cellularity and neutrophil granulopoiesis with giant megakaryocytes in ET **(a)** contrasting a moderate increase of both features in initial (prefibrotic) IMF **(b)** in patients of the same age. Erythroid precursors reveal normal-sized islets in ET **(c)**, opposed to a reduction in size and number in (prefibrotic) IMF **(d)**. a, b, naphthol-AS-D-chloroacetate esterase reaction; c, d, Ret40f immunostaining. x 170

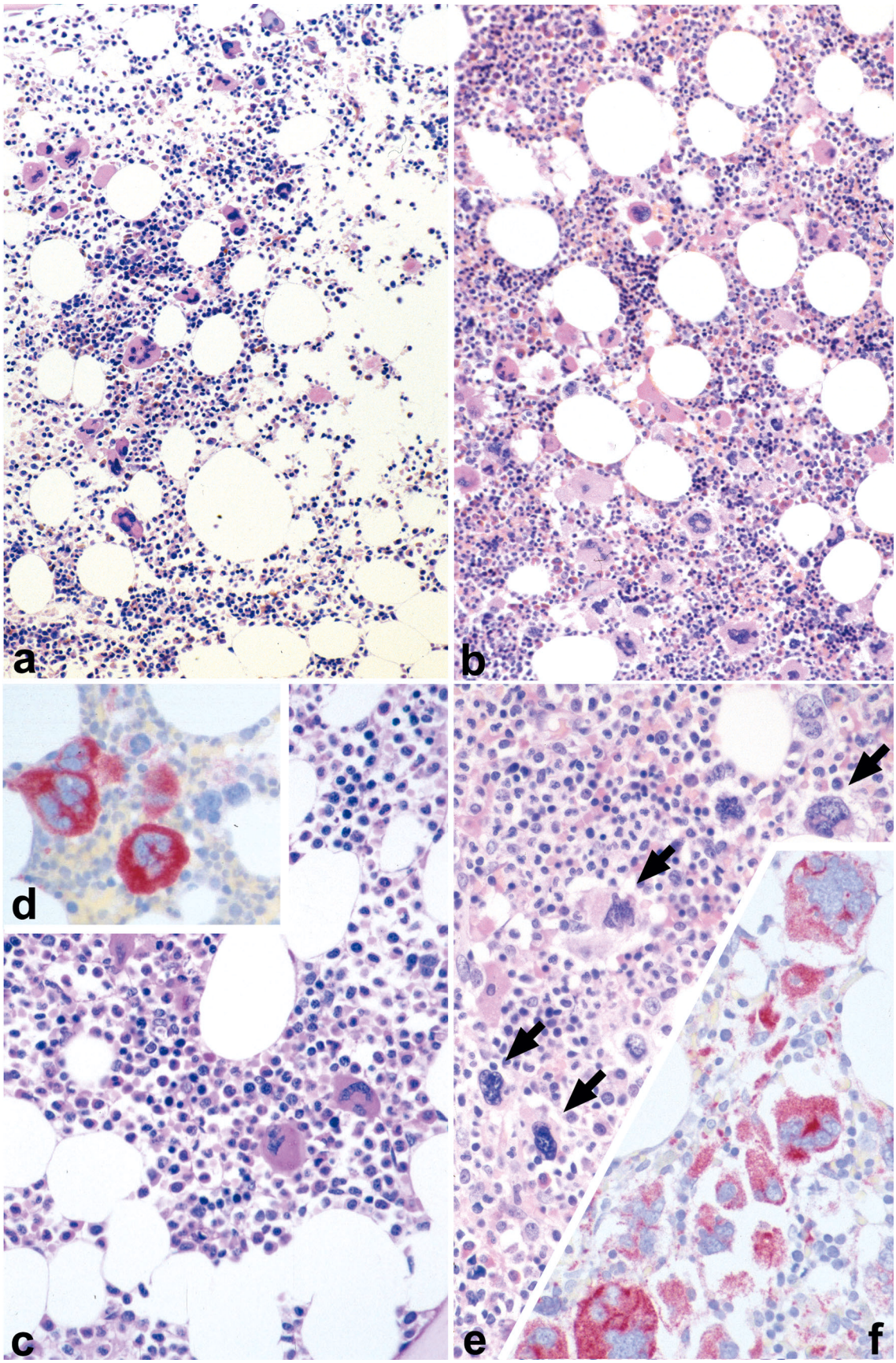

Fig. 2.
Megakaryopoiesis. Small and loose clusters of large to giant megakaryocytes characterize ET **(a)**. Contrasting this appearance initial (prefibrotic) IMF is characterized by a more dense grouping of large to small megakaryocytes **(b)**. Megakaryocytes in ET reveal deeply lobulated (staghorn-like) nuclei surrounded by a normal portion of mature cytoplasm **(c, d)**. Megakaryocytes in IMF display an abnormal cytological differentiation **(e)** with hypolobulated, cloud-like nuclei (arrows) and many small and also a few giant dysplastic (bizarre) cells showing poorly segmented nuclei with maturation defects **(f)**. a, b, e, H&E; c, PAS; d, f, CD61 immunostaining. a, b, x 170; c, e, x 380; d, f, x 570

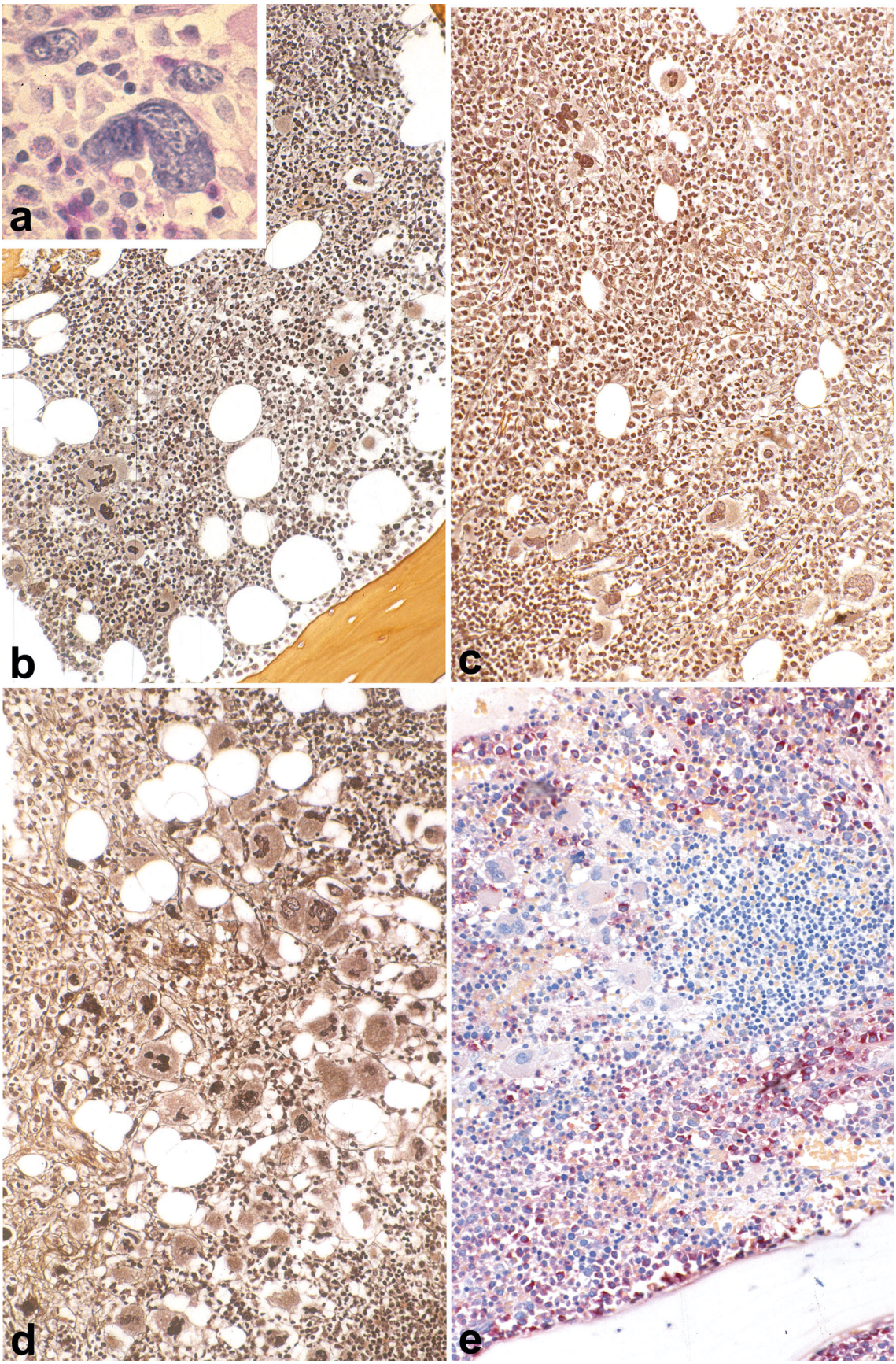

Fig. 3. Early IMF often shows naked nuclei **(a).** Compared to ET without reticulin fibres but large to giant megakaryocytes **(b)**, no increase in reticulin is recognizable in initial-prefibrotic **(c)** and a mild fibrosis in early IMF that may be associated with clusters of abnormal megakaryocytes **(d)**. Especially in initial-prefibrotic IMF well-marginated lymphoid nodules may be observed (e). a, PAS-reaction; b-d, Gomori's silver impregnation; e, naphthol-AS-D-chloroacetate esterase reaction. a, x 380; b-e, x 170

Clinical and haematological findings were in support of this classification, because they exhibited a number of parameters of distinctive impact between ET and IMF-1 (Table 4). Differences between ET and IMF-0 were more subtle, however, also recognizable by regarding certain constellations of findings that included anaemia, splenomegaly, LAP and LDH. Contrasting the platelet count that failed to exert a discriminating value in all groups, patients with IMF-0 displayed a tendency to present more frequently with a borderline to slight anaemia associated with an increased spleen size and higher LAP and LDH values compared to ET. Regarding relevant complaints, patients with ET and also IMF-0 presented more frequently (about 40%) with a history of bleeding (easy bruising) and thrombosis (about 30%) and clinically important events included also more often thromboembolic episodes and haemorrhage compared with IMF-1 patients. Development of myelofibrosis proved to be significantly different in our three categories, because during obervation time (five to almost 14 years) no patient with ET showed a relevant increase in fibres, contrasting IMF-0 with 42% patients revealing grade +1 increase and 50% patients with manifest myelofibrosis (grade +2 and +3) (Bauermeister, 1971). All IMF-1 patients displayed a progression to overt myelofibrosis that was partially associated with osteosclerosis. Moreover, after more than seven years five patients with IMF developed a blastic crisis, but none of those with ET. Survival analysis revealed significant ($p<0.01$) differences in 10-year relative survival in ET (0.97) versus prefibrotic (0.87) and early (0.74) IMF. Accordingly loss of life expectancy was 1% in ET opposed to 21% in prefibrotic and 32% in early IMF with thrombocythaemia.

Table 4. Clinical findings (mean ± SD) following discriminant analysis of bone marrow features into three different categories in 272 patients with the presumptive diagnosis of ET according to the updated PVSG criteria.

CLASSIFICATION	ET	IMF-0	IMF-1
No. of patients	98	136	38
Erythrocytes (x 10^{12}/l)	4.9±0.6	4.9±0.9	4.6±0.7
Haemoglobin (g/dl)	14.2±1.6*	14.0±1.9	12.8±2.7
Haematocrit (%)	42.8±9.6*	42.5±5.7	39.3±5.8
Leucocytes (x 10^{9}/l)	10.6±3.7	12.3±0.9	12.4±4.5
Eosinophils (%)	3.1±2.8	3.0±2.7	2.5±1.7
Basophils (%)	1.4±1.3	1.2±1.0	1.7±1.6
Myeloblasts (%)	0*	0	0.2±0.8
Erythroblasts (%)	0*	0	0.2±0.5
Thrombocytes (x 10^{9}/l)	1,184±129	1,082±430	1,462±181
LAP**	55±33*	102±78	113±76
LDH (U/l)	264±116*	272±95	595±1.2
Spleen size***	0.2±0.7*	0.7±1.1	1.2±1.3

*: parameters of distinctive impact ($p\leq 0.05$-0.0001) between ET and IMF-1. **: leucocyte alkaline phosphatase normal score 10-80. ***: cm below costal margin.

Discussion

In haematopathology discriminant analysis of bone marrow pathology that is based on histochemical staining has gained little attention so far. Although complex features of histopathology certainly warrant a systematic and more elaborate evaluation involving a large number of specimens processed by an identical technique, pathologists are generally reluctant to employ this method. Considering the apparent shortcomings of a merely descriptive approach towards the various bone marrow disorders under discussion, ET is an outstanding example. It has been stated that currently ET is not a cytogenetically or morphologically defined disease entity, and that diagnosis refers to a chronic non-reactive thrombocythaemic disorder which is not accounted for by another MPD (Tefferi et al., 1995). The impression of failing or at least ambiguous diagnostic criteria regarding bone marrow morphology may probably be related to the fact that the other subsets of MPDs may occasionally mimic ET in their clinical presentation (Buhr et al., 1992; Thiele et al., 1999a, 2000). A more recently raised question concerns the clear-cut distinction between ET and initial-early IMF with accompanying elevation of the platelet count, because significantly different survival patterns that are of clinical importance have been reported (Rozman et al., 1991; Mesa et al., 1999). Regarding ET, in a first attempt to employ discriminate analysis on routinely-stained bone marrow samples in 93 patients of the Italian ET Study Group, cluster calculation of morphological features revealed two distinctive patterns characterized by different survival rates (Annaloro et al., 1999). The first pattern involved 40 patients with an increased reticulin content, an enhancement in the numbers of myeloid precursors with defects of maturation, decreased levels of erythroid precursors and dysplastic megakaryocytes trapped in a fibrous network. In comparison with our results and in agreement with previous studies (Thiele et al., 1999a, 2000, 2002), these features are presumably consistent with initial-early IMF. For this reason, it is not astonishing that in these patients survival was calculated to be significantly shortened, opposed to the second group that failed to show an increase in reticulin fibres or lacked conspicuous abnormalities of the myeloid series and megakaryocytes (Annaloro et al., 1999). The latter cohort seems to be compatible with the first group of patients (true ET) in our study. Contrasting this rather crude descriptive analysis that included H&E staining and only three grades (decreased, normal, increased) to categorize histopathology (Annaloro et al., 1999) the semiquantitative grading presented in this investigation was more detailed. In particular attention was focused on cytological anomalies of the megakaryocytic lineage (Table 1).

Although histochemical staining methods and bone marrow biopsies were primarily employed, the results of our study are easily applicable on routinely - (H&E)

processed biopsy specimens (Fig. 2a,b,e). This feature regards explicitly the abnormalities of megakaryopoiesis as the most prominent parameters of distinction between the three groups of patients (Tables 2, 3). In this context it is noteworthy that a number of clinical studies on ET which included a bone marrow biopsy as criterion for entry reported an incidence of reticulin fibrosis ranging between 30 to 50% and an increase in collagen of about 8% (Bellucci et al., 1986; Van de Pette et al., 1986; Hehlmann et al., 1988; Murphy et al., 1997; Annaloro et al., 1999). When following the diagnostic guidelines of the PVSG (Tefferi et al., 1995; Murphy et al., 1997) these data are not significantly different from the corresponding findings of this evaluation and therefore suggest a heterogenous pattern of bone marrow pathology. In this context, the discriminatory impact of megakaryocyte anomalies that are usually encountered in IMF (Georgii et al., 1990, 1998; Buhr et al., 1992; Thiele et al., 2000, 2002) but not in ET has to be emphasized. It is mandatory that an unequivocal diagnosis of ET including the separation from the other eventually thrombocythaemic subtypes of MPDs with a significantly different prognosis warrants a representative biopsy sample. This postulation is a basic requirement for entry into any ongoing or prospective clinical trial to avoid a disturbing diagnostic inconsistency. Moreover, the generally accepted criteria of the PVSG (Tefferi et al., 1995; Murphy et al., 1997) are in need of an amendment and modification which has already been initiated (Michiels and Juvonen, 1997; Imbert et al., 2001). First of all, this improvement should discard the rather ill-defined criterion of "no collagen fibrosis or <1/3 biopsy area without both marked splenomegaly and leuko-erythroblastic reaction" (Murphy et al., 1997). Instead, as a positive finding, in addition to a sustained raise of the platelet count exceeding at least $400x10^9$/l (Lengfelder et al., 1998; Sacchi et al., 2000), histopathology of the megakaryocytic lineage should be introduced as a major point of diagnosis. In accordance with the results derived from this study the following criteria are suggested and have generally been regarded by the recently published WHO-classification (Imbert et al., 2001): no relevant increase in reticulin fibres or overall cellularity, but a prominent proliferation of the megakaryocyte lineage showing increased numbers of large to giant megakaryocytes without maturation defects (i.e. bulbous nuclei, abnormal nuclear lobulation, hyperchromasia). However, it should be emphasized that there is certainly a compelling need to perform a prospective multicentre and international clinico-pathological study on ET to validate this concept of a distinct histopathology characterising this disorder, as an invaluable aid for differentiation from thrombo-cythaemias complicating other MPDs.

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