

## Review

# Diagnostic impact of bone marrow histopathology in polycythemia vera (PV)

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**Summary.** The criteria of the Polycythemia Vera Study Group (PVSG), although acknowledged as the gold standard to establish the diagnosis of polycythemia vera (PV), do not regard bone marrow (BM) histopathology. Arguments include the existence of sufficient objective markers of disease and the lack of independently performed morphological studies or standardized criteria. The aim of this review is to evaluate morphological characteristics of erythrocytosis and to determine whether distinctive patterns of histopathology exist. A review of the pertinent literature and evaluation of 334 patients from our files with a borderline to marked increase in hemoglobin was performed. In extension to former descriptions of BM features by the PVSG, a tri-lineage myeloproliferation (panmyelosis) with a pleomorphic appearance of megakaryopoiesis revealed that, besides increase in size, there was a lack of gross cytological anomalies. Differentiation from secondary polycythemia (SP) was accomplished by regarding these features and the conspicuously expressed stromal changes (plasmacytosis, eosinophils, cell debris and iron deposits). In about 96% of this cohort a clear-cut separation from SP was achieved, even in the initial (latent) stages. When accompanied by an elevated platelet count, these precursor stages may clinically mimic essential thrombocythemia because they are not recognized by the conventional criteria. Advanced stages (spent phases) of PV were consistent with an increased left-shifted granulocytic proliferation, accompanied by reduction of erythroid precursors and progressive myelofibrosis (post-polycythemic myeloid metaplasia). Finally, an increase in dysplastic changes and immaturity signalled a transition into blastic crisis.

In conclusion, PV is characterized by a distinctive pattern of histopathology that has been gained in an independent and blind fashion and therefore, dissolves arguments about failing specificity.

**Key words:** Polycythemia vera, Secondary Polycythemia, Histopathology, Megakaryopoiesis, Stromal changes, Discriminant analysis, Bone marrow biopsies

### Introduction

In a recently published, very comprehensive review on polycythemia rubra vera (PV), it has been stated that bone marrow (BM) examinations are not part of the diagnostic criteria (Spivak, 2002). Major arguments include the existence of sufficient clear objective markers of the disease which make the involvement of histopathology unnecessary and that only patients with already clinically established diagnosis of PV have been studied so far (Pearson and Messinezy, 1996; Pearson, 1998). This rather critical attitude towards the diagnostic impact of BM features, characterizing this subtype of chronic myeloproliferative disorders (CMPDs), is probably based on several, mostly historically derived assumptions. Accordingly, the diagnosis of PV is usually performed by the world-wide accepted classical and updated standards proposed by the Polycythemia Vera Study Group (PVSG) which do not include BM morphology (Berlin, 1975; Berk et al., 1986; Bilgrami and Greenberg, 1995; Pearson and Messinezy, 1996; Pearson, 1998; Messinezy and Pearson, 1999; Spivak, 2002). Histological evaluation of BM biopsies, studied quantitatively in the PVSG trial, revealed cellular hyperplasia with loss of fat cells, an increase in megakaryocytes, a significant reduction of iron deposits and a slight to marked reticulin myelofibrosis in more than one third of specimens (Ellis et al., 1975, 1986; Ellis and Peterson, 1979). It is reasonable to assume (Spivak, 2002) that the described features can never alone be diagnostic for the establishment of PV or serve as tool for a clear-cut discrimination from reactive (secondary-spurious) variants of polycythemia (SP). As has been appropriately emphasized, the challenge to use histopathology as a reliable criterion implies a more accurate definition of standardized and readily to

reproduce parameters of discriminating impact (Pearson, 1998). On the other hand, concerning PV in the new WHO classification, histopathology was entered as a minor point for diagnosis, together with the determination of the erythropoietin (EPO) level and endogenous erythroid colony (EEC) formation in vitro (Pierre et al., 2001). This advance in the recognition of less essential diagnostic criteria, in addition to the classic ones, is also reflected by results derived from a nationwide survey of practice patterns among the American Society of Hematology (ASH) members (Streiff et al., 2002). Moreover, new methods of processing and evaluating trephine biopsies of the BM that have been achieved in the last two decades (Dickstein and Vardiman, 1993; Georgii et al., 1998; Thiele et al., 1999b; Michiels and Thiele, 2002) were not always acknowledged to their full extent. Contrasting major diagnostic criteria (red cell mass, hemoglobin-hematocrit level, absence of cause of secondary erythrocytosis, palpable splenomegaly), EEC techniques for the demonstration of EPO-independent erythroid colony formation (Lemoine et al., 1986; Eridani et al., 1987; Dudley et al., 1989; Weinberg et al., 1989; Juvonen et al., 1993) are cost-effective, time-consuming and of limited value, because they are not standardized or generally available. The development of a sensitive and specific assay for the EPO is more important in this regard. While a significantly elevated EPO level suggests tissue hypoxia as the (reactive) cause for erythrocytosis, a normal or slightly raised value does not exclude this pathomechanism (Birgegard and Wide, 1992; Westwood et al., 1993; Messinezy et al., 1995; Carneskog et al., 1998). Consequently, serum EPO estimation would be preferred to EEC studies by most investigators because it is more reproducible and less expensive.

Finally, over-expression of the mRNA of a novel gene, designated as PRV-1, was identified in mature peripheral blood neutrophil leukocytes and hailed with great enthusiasm as a molecular marker of PV (Temerinac et al., 2000; Pahl, 2002; Klippel et al., 2003; Pahl, 2003a,b, 2004). However, these expectations were deflated by the demonstration that PVR-1 is constitutively expressed in BM cells and therefore, does not discriminate PV from reactive and other CMPDs (Bock et al., 2003). This statement was supported by the finding that some patients with both, EPO-independent EEC growth and clinical as well as laboratory characteristics of PV, failed to exhibit raised mRNA levels of PVR-1 (Kralovics et al., 2003; Liu et al., 2003). Moreover, in a prospective study, real-time PCR-based assays showed a PVR-1 expression across the CMPDs and also in SP and therefore it was concluded that quantifying neutrophil PRV-1 mRNA is not self-sufficient for the diagnosis of PV (Tefferi et al., 2004). Finally, in about 50% of patients with essential thrombocythemia (ET), diagnosed according to the PVSG criteria (Murphy et al., 1997), elevated levels of PVR-1, together with EEC formation, were described (Teofili et al., 2002; Pahl, 2003b; Griesshammer et al.,

2004) and associated with a higher risk of developing vascular complications (Johansson et al., 2003). However, as outlined in a recently published, comprehensively conducted study, elevated neutrophil mRNA levels of this gene are no specific markers for the diagnosis of PV or any other CMPD, but overexpression reflects a rather abnormal granulocyte production and/or release (Passamonti et al., 2004).

In consideration of these unconventional diagnostic parameters that are limited in usage and often impaired by ambiguous results (Westwood et al., 1993), a general revision of the diagnostic criteria for PV has been proposed (Michiels and Juvonen, 1997). Amongst others, this critical appraisal should include an elaborate review of BM morphology that is certainly warranted to test its specificity and discriminating impact concerning the differentiation of erythrocytosis. In this context, it is important that this assessment of histopathology is to be based on a more refined handling and work-up of the corresponding tissue samples (Bartl et al., 1993; Georgii et al., 1996, 1998; Thiele et al., 1999b, 2001a). Additionally, processing should include not only Giemsa stain, but also enzyme histochemistry (i.e., naphthol AS-D-chloroacetate esterase reaction - Leder stain, periodic acid Schiff reagent - PAS) to accommodate a more scrutinized evaluation of all three major hematopoietic cell lineages (neutrophil granulo- versus erythropoiesis and megakaryocytes). Furthermore, to cope with the argument of standardized criteria of distinctive value (Pearson and Messinezy, 1996; Pearson, 1998), an appropriate semi-quantitative grading and subsequent discriminant analysis must be carried out (Thiele et al., 2001b). These considerations are motivating points, to be followed in patients presenting with a mild to significant erythrocytosis of unknown origin, with the explicit aim of initiating a re-awakening interest in BM histopathology in these disorders.

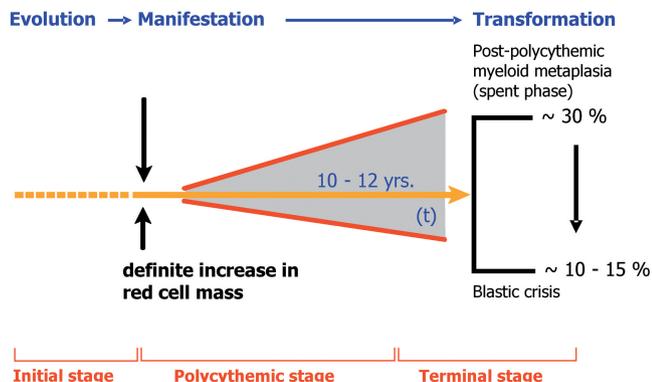
Since this problem remains an open and controversial question, we have revised the pertinent literature in this field and re-evaluated our filed BM specimens together with the corresponding clinical records comprising more than 334 patients with a borderline to overt sustained erythrocytosis (hemoglobin: > 17g/dl in 171 males and > 15g/dl in 163 female patients with a median age of 60 years). However, for a more stringent discussion of relevant BM findings, it is mandatory to realize firstly, the peculiar dynamics of the disease process in PV which is shown in Fig. 1.

#### *Initial stage of PV*

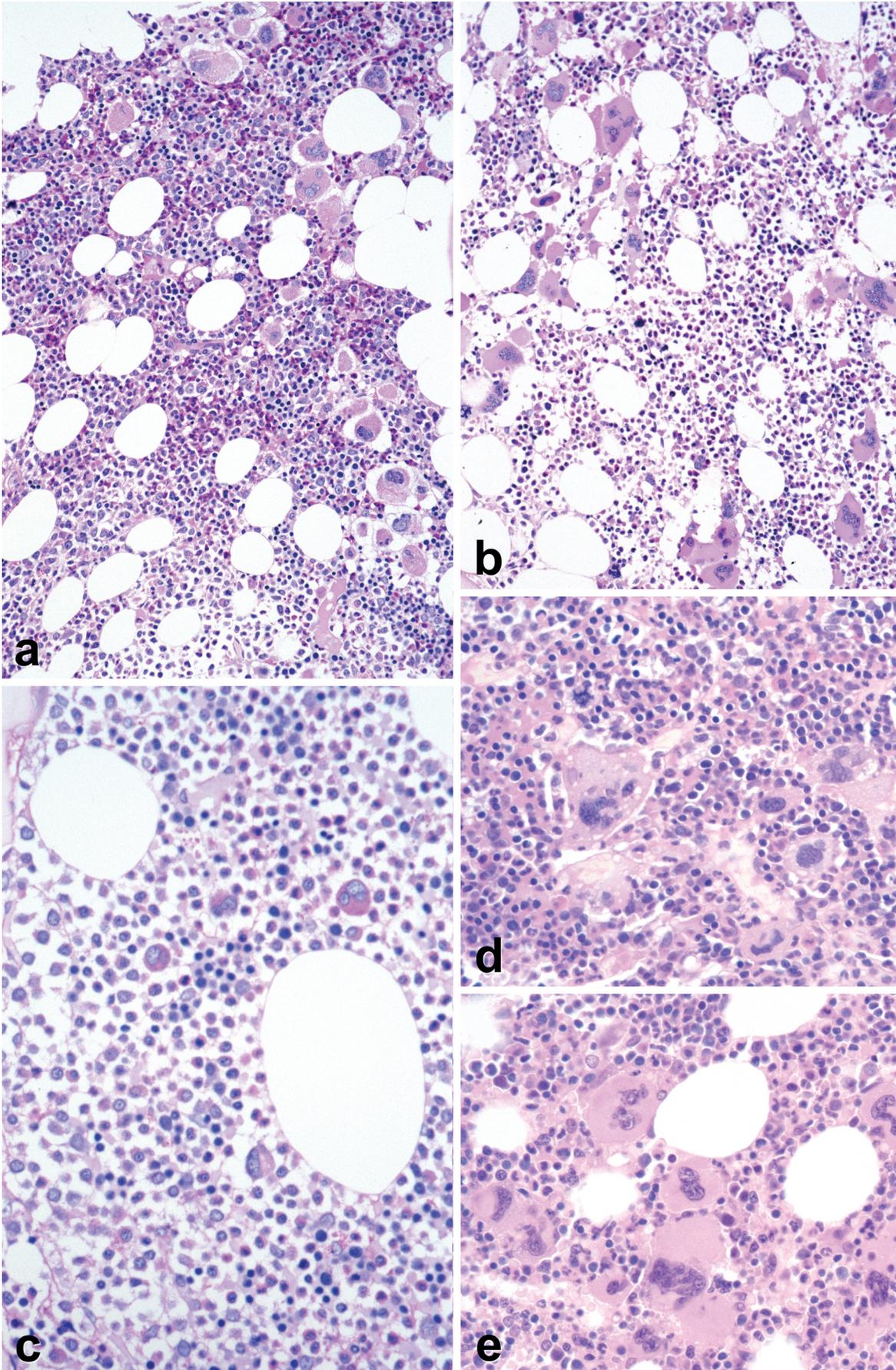
Precursor stages of PV are, by definition, not presenting with a significant increase in the red cell mass or hemoglobin/hematocrit level and therefore, are not conforming with neither the classical nor updated diagnostic criteria of the PVSG (Berlin, 1975; Pearson and Wetherley-Mein, 1979; Bilgrami and Greenberg, 1995; Pearson and Messinezy, 1996; Pearson, 1998; Messinezy and Pearson, 1999; Murphy, 1999; Spivak,

2002; Tefferi, 2003) or the new WHO criteria (Pierre et al., 2001). Although PV onset is difficult to recognize, it is tempting to assume that a number of patients, formerly described as idiopathic (pure, benign) erythrocytosis (Pearson and Wetherley-Mein, 1979; Najean et al., 1981; Berglund and Zettervall, 1992; Messinezy et al., 1994; Pearson, 1998), may be included in the group of initial PV that occasionally presents with an elevated platelet count and thus may mimic ET (Michiels and Thiele, 2002). This point is supported by the findings of an Italian study group who recorded that thrombotic events may be frequently encountered in the years preceding the establishment of the diagnosis of manifest PV by conventional criteria (Gruppo Italiano Studio Policitemia, 1995). Therefore, neither the PVSG nor the WHO criteria are optimal to identify patients in early stage PV. As shown in an epidemiological study on 88 patients covering about 5 years of observation (Ruggeri et al., 2003), the rate of progression of mild erythrocytosis to idiopathic erythrocytosis (11 patients) and manifest PV (PVSG criteria) is relatively low (total 5 patients). One of the most interesting points in these patients with a sustained, yet only borderline elevation of the red cell mass, are cases who present with an excess of platelets ( $> 500 \times 10^9/l$ ), suggesting ET. In the original series of patients included in the PVSG trial, differentiation between PV and ET seemed to be difficult in a number of cases that apparently exhibited a transition between these two conditions (Iland et al., 1987; Murphy et al., 1997). These shortcomings in separating ET from PV are evident when applying modern techniques like EEC studies and determinations of PVR-1 expression, particularly in those patients that do not fulfill all diagnostic requirements of PV (latent stages). Transformation of so-called ET in PV was observed in a considerable number of patients (Jantunen et al., 1999), especially when including ECC studies (Shih and Lee, 1994). Since approximately 50% of patients with so-called ET displayed ECC formation together with an elevated PVR-1 level, a heterogeneity of ET was postulated (Teofili et al., 2002; Pahl, 2003b;

Griesshammer et al., 2004). In this context, it has been stated that PRV-1-positive ET patients comprise a pathophysiologically distinctive subgroup that is at risk for the development of complications (Johansson et al., 2003) and for the emergence of PV (Griesshammer et al., 2004). On the other hand, some caveats have to be taken into account because opposed to the meticulously conducted clinical studies in combination with cell culture and molecular biological marker profiles, the descriptions of BM histopathology in these patients by no means match these elaborate parameters. A survey of our material reveals that, in addition to 164 patients with manifest PV, according to the WHO classification (Pierre et al., 2001), 44 cases revealed initial (latent) PV. Amongst the latter, 23 patients (about 52%) presented with a platelet count in excess of  $600 \times 10^9/l$  and 4 with a thrombocytosis of  $> 1,000 \times 10^9/l$ . Therefore, at least for the unexperienced investigator, some of the patients included in this cohort may simulate ET. BM samples revealed a slightly increased cellularity and a prominent megakaryopoiesis, characterized by a variety of cell sizes (Fig. 2a) contrasting ET and a prevalence of giant to large megakaryocytes (Fig. 2b). In particular, these features of megakaryopoiesis served as means to discriminate SP from PV and ET (Fig. 2c-e), besides differences concerning cellularity and the involvement of the other hematopoietic cell lineages. On the other hand, recognition of initial PV should be based on a careful follow-up including sequential BM biopsies. In a series of 39 patients with a borderline increase in hemoglobin ( $< 18.5$  g/dl in males,  $< 16.5$  g/dl in females), i.e. values that are not consistent with the accepted diagnostic guidelines of PV (Pierre et al., 2001; Spivak, 2002), histopathology showed distinctive features that were clearly discriminable from SP (Thiele et al., 2001b). It should be added that all patients with initial PV were treated only by phlebotomy and later developed manifest stages with symptoms and signs fulfilling the classic clinical criteria. Usually early stage (latent) PV is characterized by a minimal to slight increase in cellularity and an involvement of all three major hematopoietic cell lineages (Fig. 3a), a feature that contrasts definitively SP (Fig. 3b). Discriminant analysis (Everitt and Dunn, 2001) of more than 20 standardized BM characteristics (semi-quantitative grading) revealed that an increase in megakaryocyte size with an occurrence of large to giant cells containing hyperlobulated nuclei (Fig. 2c versus 2d), as well as an inflammatory stroma reaction (perivascular plasma cells, accumulation of debris, eosinophils, iron deposits) and hypercellularity were the most important parameters (Fig. 3c-f). In extension to these previous studies on a small series of patients, a blinded and independently performed evaluation of standardized BM features was performed in 334 patients with borderline to overt erythrocytosis. Semiquantitative analysis and discriminating relevance of these parameters served as means to distinguish reactive (SP) from early-initial autonomous lesions (PV). According to this procedure, subsequent revision of clinical records including

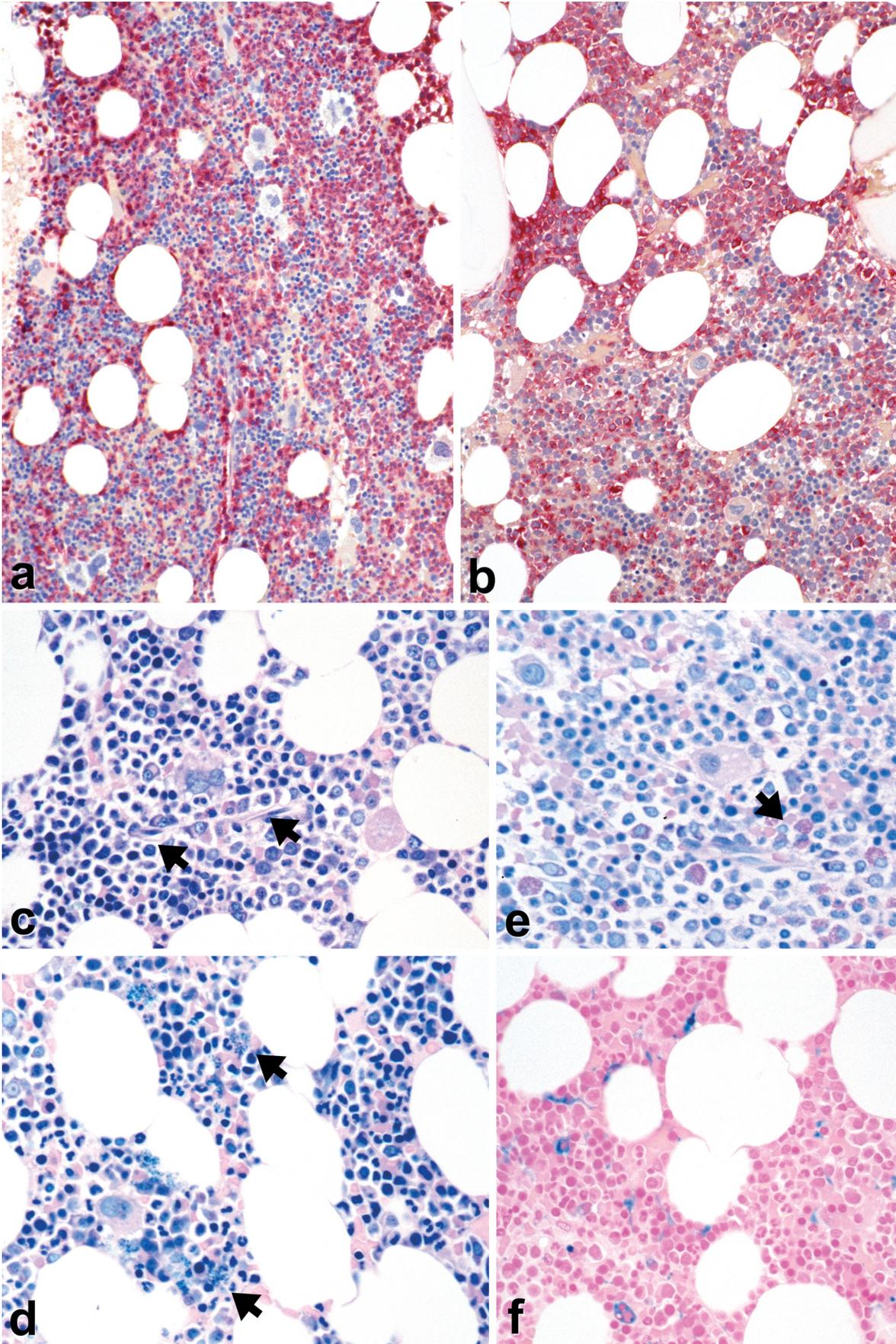


**Fig. 1.** Dynamics of the disease process in PV including the latent (subclinical) and terminal (blastic) stages.

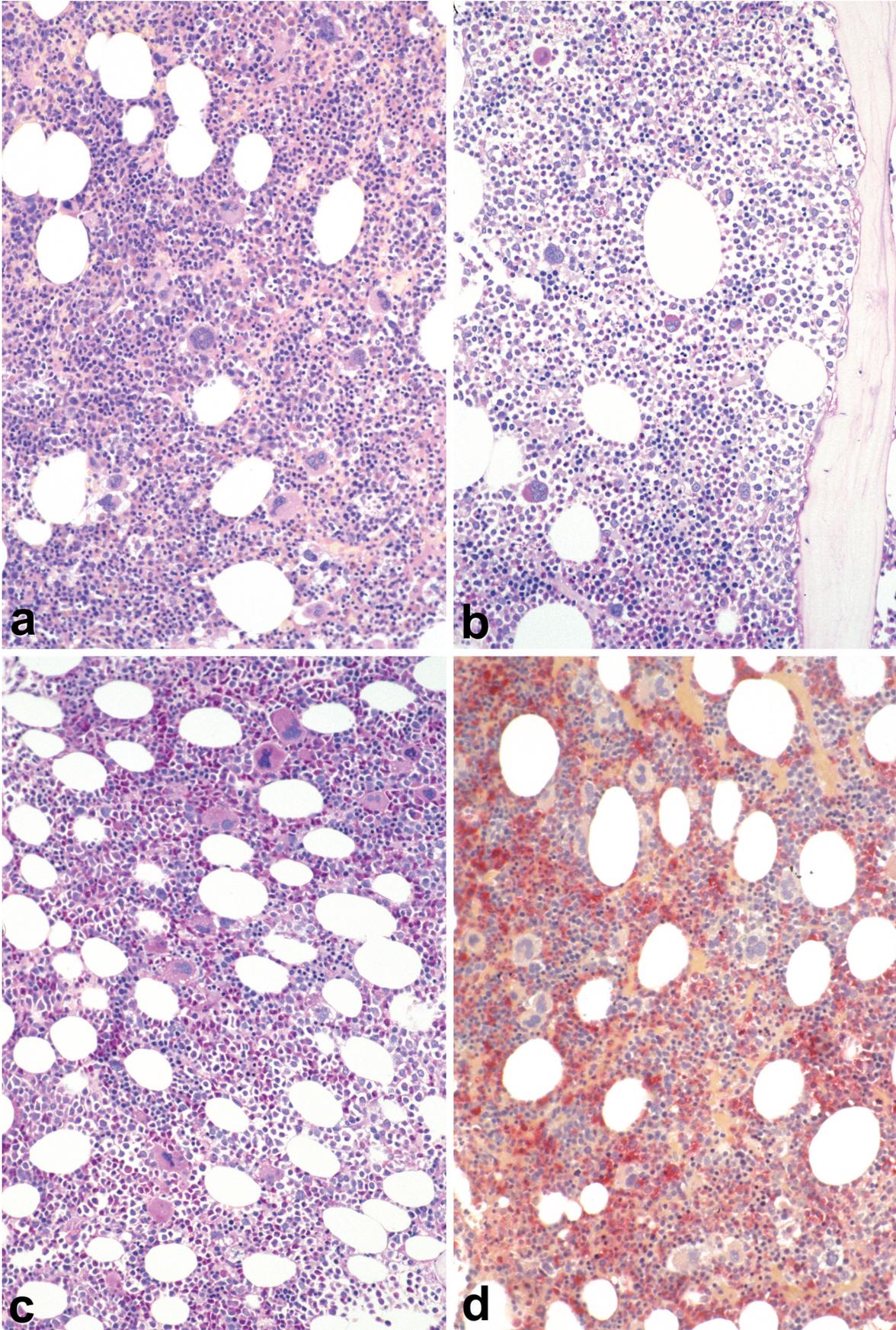


**Fig. 2.** Initial (latent) PV presenting with an elevated platelet count versus ET and SP. **a.** Early PV with a prominent growth of large to small megakaryocytes and slight increase in cellularity. **b.** ET shows a prevalence of large to giant megakaryocytes and no increased cellularity. **c.** Megakaryocytes are small in SP, contrasting the wide range of sizes that generates a markedly expressed pleomorphic aspect in PV (**d**) when compared to a predominance of large to giant forms in ET (**e**). PAS (periodic acid Schiff reagent). a, b, x 180; c, d, e, x 380

*Bone marrow in polycythemia vera*



**Fig. 3.** Early PV versus SP. **a.** Increased cellularity and involvement of all three hematopoietic cell lineages characterize PV opposed to SP (**b**). On the other hand, stromal changes are prevalent in SP (arrows) such as perivascular arrangement of plasma cells (**c**), accumulation of cell debris (**d**), dispersed eosinophils (**e**) and finally, iron-laden macrophages (**f**). **a** and **b**, AS-D-chloroacetate esterase; **c**, **d**, and **e**, Giemsa stain; **f** Perls' reaction. **a**, **b**, x 180; **c**, **d**, **e**, **f**, x 380



**Fig. 4.** Manifest (polycythemic) PV versus SP. An overall increased cellularity is present in full-blown PV (a), however, may occasionally be found in reactive states (b). Megakaryocytes reveal conspicuous differences in both conditions by showing wide ranges of sizes in PV (c) besides the prominent erythroid proliferation and prominent neutrophil granulopoiesis (d). a, b, H&E; c, PAS (periodic acid Schiff reaction); d, AS-D-chloroacetate esterase. x 180

## Bone marrow in polycythemia vera

laboratory data, EPO level and follow-up, 96% of the patients were placed into the correct category, including PV in 208 and SP in 113 patients. It is necessary to emphasize that some of these variables, but mainly increased cellularity and large to giant megakaryocytes are amongst other findings generally regarded as characteristics for full-blown (polycythemic) PV (Kurnick et al., 1972; Ellis et al., 1975; Bartl et al., 1993; Georgii et al., 1996, 1998; Thiele et al., 2001a). At this initial stage of PV, in addition to histopathology, only results of cell culture studies with demonstration of EEC growth (Lemoine et al., 1986; Eridani et al., 1987; Partanen et al., 1989) or a determination of the EPO level (Birgegard and Wide, 1992; Westwood et al., 1993; Messinezy et al., 1995; Carneskog et al., 1998) may offer a diagnostic clue as to the nature of this condition presenting with only a borderline to moderate erythrocytosis. Persuasive evidence has been produced, demonstrating that histopathology of the BM is a very effective way of establishing PV at onset in this cohort of patients (Thiele et al., 2001a) who so far have been neglected when strongly adhering to the gold standards of clinical diagnosis. Consequently, a re-consideration of the indication for performing a BM biopsy in patients with a sustained erythrocytosis of unexplainable origin is necessary. In this context one should mention that the term erythrocytosis has some advantages over polycythemia to describe patients with a raised hemoglobin/hematocrit value and therefore deserves to be widely applied (Messinezy and Pearson, 1999).

### Polycythemic stage of PV

Generally, histopathology of manifest PV is

**Table 1.** Relative frequency and ranking (discriminating relevance) of standardized bone marrow features (semi-quantitative evaluation: -, < 20%; +, ≥ 20%) in erythrocytosis (hemoglobin: > 17 g/dl in men and >15 g/dl in women, corresponding to a hematocrit > 48 % and > 43%).

	PV	SP
Megakaryocytes		
large to giant size	+	-
pleomorphic aspect (differences in size)	+	-
Stroma		
perivascular plasmacytosis	-	+
increased cellularity	+	-
presence of cellular debris	-	+
iron-laden macrophages	-	+
Increased eosinophils	-	+
Megakaryocytes		
increased quantity	+	-
increased nuclear lobulation	+	-
loose clustering	+	-
naked nuclei	+	-
Stroma		
lymphoid nodules	+	-
increased reticulin	+	-

PV: polycythemia vera; SP: secondary polycythemia. Predicted group membership: > 96%.

characterized by a trilineage proliferation (so-called panmyelosis) involving erythroid precursors, megakaryocytes and neutrophil granulopoiesis, however, to a different degree (Kurnick et al., 1972; Bartl et al., 1993; Dickstein and Vardiman, 1993; Georgii et al., 1996, 1998; Thiele et al., 1999b, 2001a; Michiels and Thiele, 2002). Consequently, although hypercellularity in relation to age-matched hematopoiesis (Fig. 4a, c, d) is a common feature, as shown in a series of 164 patients with manifest (polycythemic) PV, according to the WHO criteria (Pierre et al., 2001), it may occasionally also be encountered in SP (Fig. 4b). Contrasting this finding in the biopsy material of the PVSG trial, 13% of the patients revealed a normal amount of hematopoiesis (Ellis et al., 1975, 1976; Ellis and Peterson, 1979). Consequently, one should be aware that in the elderly population comprising PV patients (median age 60 years), the subcortical (superficial) BM spaces are usually occupied by fat cells and any expansion of hematopoiesis towards this area implies hypercellularity. For this reason, a representative trephine biopsy, performed at an orthograde direction, is needed for an accurate assessment. For an easy recognition and quantification of neutrophil granulopoiesis versus erythropoiesis a special stain like naphthol-A-SD-chloroacetate esterase (Fig. 4d) or myeloperoxidase is recommended which is superior to the routine application of hematoxylin-eosin (HE). Following this procedure, it is apparent that the normally small and rounded islets of nucleated erythroid precursors show a conspicuous enlargement and a tendency to merge into sheets (Fig. 4d). Although these changes are significantly more pronounced in PV (Thiele et al., 1993), they may be also expressed in a few cases with severe SP and therefore, are not very reliable diagnostic parameters. Similar features may be observed regarding the neutrophil cell lineage whereby an increase in pro- and metamyelocytes (left-shifting) is frequently displayed in both entities. On the other hand, megakaryocytes have been acknowledged to exhibit characteristics that enable a distinction between PV and other subtypes of CMPDs and reactive states as well (Georgii et al., 1998; Thiele et al., 2001a; Michiels and Thiele, 2002). In significant extension to former evaluations of the PVSG (Ellis et al., 1975), in which 95% of the biopsies demonstrated only an increase in megakaryocyte number, cytological appearance exerts a discriminating impact. It has been repeatedly emphasized that megakaryopoiesis in early as well as in full-blown PV, displays a pleomorphic aspect (Figs. 2a,d; 3a; 4a,c), i.e., small, medium sized, large and giant megakaryocytes are either dispersed or loosely clustered (Thiele et al., 1988, 2001,b; Georgii et al., 1990, 1996, 1998; Buhr et al., 1992). In particular, the giant megakaryocytes with their hyperlobulated nuclei that fail to show abnormalities (deviation from nuclear-cytoplasmic maturation in addition to size), may serve as diagnostic hallmark (Fig. 4a, c) contrasting the small to medium-sized ones in SP (Fig. 4b). In PV patients with an excess of platelets, differentiation from ET is possible

by regarding the histological pattern of left-shifted (immature) erythroid and granulocytic proliferation (Fig. 2a-e) associated with a megakaryopoiesis that exhibits a striking variety of cell sizes (Georgii et al., 1998; Thiele et al., 1999a,b).

Discriminate analysis (Everitt and Dunn, 2001) reveals that in addition to the peculiar appearance of megakaryocytes, certain constituents of the stroma compartment enable a clear-cut distinction between PV and SP (Table 1). Iron-laden macrophages are rarely observable in PV and account for less than 6 % of patients (Ellis et al., 1975; Thiele et al., 2001a), thus contrasting the frequent occurrence of this phenomenon in SP (Fig. 3f). An increase in reticulin fibers, usually found in 10 % to 20 % of patients with PV at diagnosis, is never encountered in SP (Ellis et al., 1986; Georgii et al., 1990, 1996, 1998; Thiele et al., 1999b). Moreover, SP normally shows an inflammatory reaction with deployment of perivascular plasma cells (Fig. 3c), many scattered eosinophils and small accumulation of cell debris ingested by macrophages (Fig. 3e, d), i.e., features that are most prominent in so-called smokers polycythemia (Thiele et al., 2001a,b; Michiels and Thiele, 2002). These findings are demonstrated in Table 1 by the ranking of standardized parameters which exert a discriminating relevance. Finally, it should be noted that morphometry of BM vascularity demonstrated an increase in microvessel density with luminal distension and enhanced tortuosity, due to densely packed erythrocytes (Lundberg et al., 2000; Kvasnicka and Thiele, 2004; Panteli et al., 2004) as a prominent stromal change in PV. Recognition of all these histological patterns, that have been widely neglected in the original descriptions of BM features in patients with PV (Ellis et al., 1975, 1986; Ellis and Peterson, 1979), offers major evidence as how to separate this condition from reactive changes and also from allied subtypes of CMPDs.

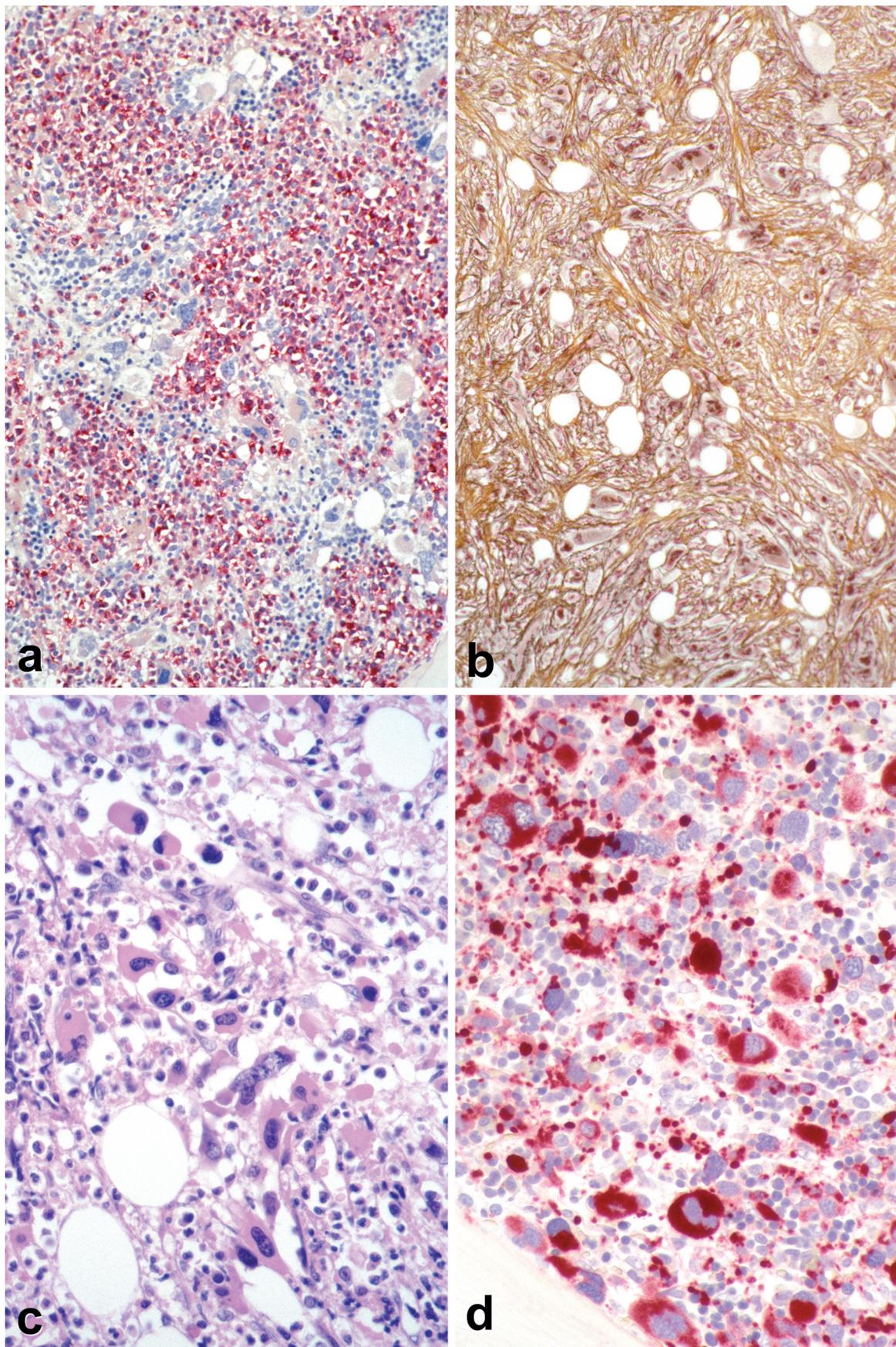
#### *Late stage PV*

Contrasting SP and manifest (polycythemic) stages of PV, advanced and terminal PV is characterized by conspicuous BM lesions as could be demonstrated in 29 patients from our series of follow-up biopsy specimens. These include a more prominent, left-shifted neutrophil granulopoiesis, associated with a reduction of nucleated erythroid precursors (Fig. 5a). Myelofibrosis begins with a thickening of sinus walls (so-called sinus wall-sclerosis), followed by a dense meshwork of reticulin and finally, development of coarse collagen bundles, progressing to a scarring throughout the BM and subsequent effacement of hematopoiesis (Fig. 5b). Ensuing reticulin-collagen fibrosis is usually accompanied by an increased proliferation of immature and atypical (dysplastic) megakaryocytes (Fig. 5c). These abnormalities of maturation may create bizarre forms of megakaryopoiesis (Fig. 5d) and may indicate an acceleration of the disease process. According to follow-up examinations with repeatedly performed BM biopsies, these terminal stages are clinically associated

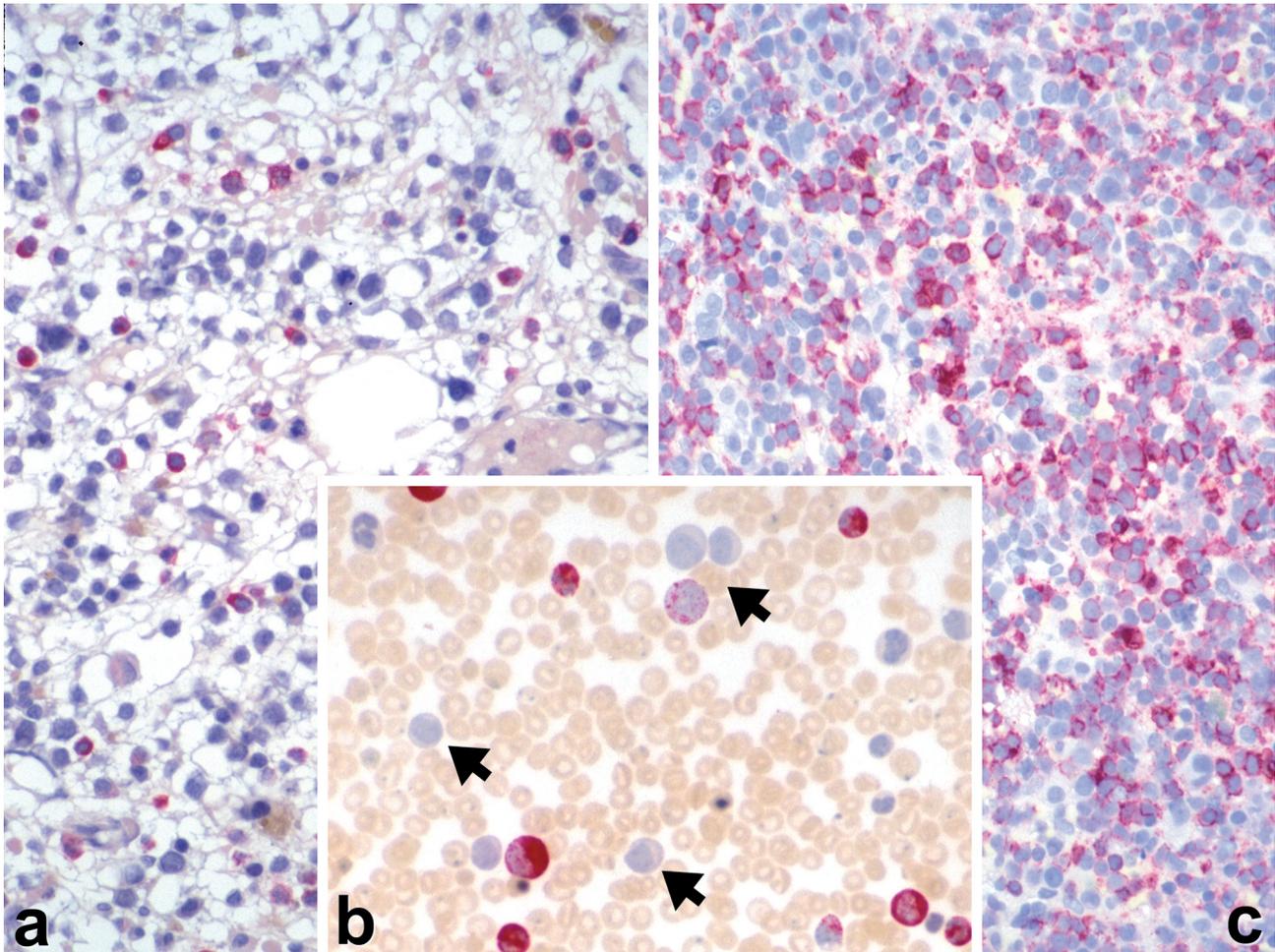
with splenomegaly and symptoms of so-called (post-polycythemic) myeloid metaplasia (PPMM), in common usage, synonymous with the spent phase of PV (Ellis et al., 1986; Murphy, 1999). This rather ill-defined phase denotes the progression of PV to a state of BM failure and consequently, may be regarded as a possible prelude to leukemia (Spivak, 2002). A latency period of eight to ten years should be assumed before onset of this complication, considered by many as an inevitable event in the natural history of this disorder and forerunner of leukemic transformation (Buhr et al., 1993; Georgii et al., 1998; Spivak, 2002; Tefferi, 2003). In this context it is noteworthy that histopathology in these terminal stages of PPMM is not distinguishable from full-blown chronic (primary) idiopathic myelofibrosis (Georgii et al., 1996). A conflict of opinion continues to persist concerning the progression of myelofibrosis, since there are only few reports relating to the frequency of this phenomenon by evaluating results of repeatedly performed trephine biopsies. Regarding dynamics of myelofibrosis in PV, one should be aware that about 10 - 20 % of patients already present with a mild to moderate (reticulin) fibrosis at onset (Ellis et al., 1986; Buhr et al., 1993; Georgii et al., 1996, 1998; Thiele et al., 1999b; Michiels and Thiele, 2002). The development of marked (collagen) myelofibrosis was found to occur in less than 20% of patients (Ellis et al., 1986; Georgii et al., 1996, 1998) and to display not only a strong time-related progression (Buhr et al., 1993; Georgii et al., 1996), but unfortunately, also a positive relationship to preceding cytostatic therapy (Lawrence et al., 1969; Landaw, 1976; Najean et al., 1981; Nand et al., 1990; Weinfeld et al., 1994; Brandt and Anderson, 1995; Michiels et al., 2000; Spivak, 2002). Acute leukemia with excess of blasts in the BM is a relatively rare event, according to sequential BM examinations, and accounts for less than 5 % (Georgii et al., 1998). However, this figure is too low compared to clinical experience which estimates a spontaneous and therapy-related incidence ranging between 10 % and 15 % (Ellis et al., 1986; Bilgrami and Greenberg, 1995; Spivak, 2002). This striking difference is explainable, because in the case of suspected leukemic transformation complicating late stage PV, clinicians are reluctant to perform a trephine biopsy. Acute leukemic transformation may be preceded by an ill-defined phase of acceleration exhibiting a gradual increase in anomalies of differentiation of single cell lineages (Fig. 5c), accompanied by a loose infiltrate of blasts (Fig. 6a). Appearance of blasts in the peripheral blood (Fig. 6b) and an effacement of hematopoiesis by a densely packed, partially CD34<sup>+</sup> blast population in the BM is in keeping with overt leukemia (Fig. 6c).

#### **Conclusion**

PV is characterized by a certain pattern of histopathology that has been gained in an independent and blinded fashion and therefore, dissolves arguments about a failing specificity of BM lesions in this disorder (Pearson, 1998; Spivak, 2002). The set of diagnostic



**Fig. 5.** Late stage PV. **a.** Increase in granulocytic proliferation with associated abnormalities of megakaryopoiesis and development of overt myelofibrosis (**b**) indicates postpolycythemic myeloid metaplasia. These changes are usually accompanied by marked maturation defects of megakaryopoiesis (**c**) with a gradual transition into bizarre forms (**d**) signalling acceleration. a, AS-D-chloroacetate esterase; b, Gomori's silver impregnation; c, PAS (periodic acid Schiff reagent); d, CD61 immunostaining. a, b, x 180; c, d, x 380



**Fig. 6.** Leukemic transformation in PV. **a.** Loose arrangements of blasts indicate an impending transformation into manifest leukemia. **b.** Appearance of blasts in the peripheral blood (arrows) and densely packed primitive CD34<sup>+</sup> cells in the bone marrow (**c**) are in line with overt leukemia. **a.** AS-D-chloroacetate esterase; **b.** Myeloperoxidase; **c.** CD34<sup>+</sup> immunostaining. **a, c,** x 380; **b,** x 1080

criteria with discriminating capacity is listed in Table 1. Sensitivity is high in our material (96%) which includes more than 334 patients with erythrocytosis comprising 208 patients with initial to full-blown (polycythemic) states of PV. It has been shown that examination of BM specimens not only enhances diagnostic reliability, in particular when considering the precursor stages that are not covered by the diagnostic criteria of the PVSG and WHO, but also enables the recognition of evolving myelofibrosis (spent phase) and insidious leukemic transformation. Consequently, in addition to the well-accepted clinical standards, BM histopathology should be included as a major point of diagnosis for entry into any prospective study or clinical trial on all variants of erythrocytosis.

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