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Stem Cell Disorders

Relevance of bone marrow features in the differential diagnosis between essential thrombocythemia and early stage idiopathic myelofibrosis

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ABSTRACT

Background and Objectives. **Diagnosis of essential thrombocythemia (ET) remains a challenging problem and has been predominantly established by exclusion of other thrombocythemic disorders. In this context the updated diagnostic criteria of the Polycythemia Vera Study Group (PVSG) are generally accepted, although histopathologic features of the bone marrow were only marginally considered.**

Design and Methods. **A retrospective evaluation was performed of 168 patients presenting with ET in accordance with the criteria of the PVSG. Analysis was focused on the discriminating impact of bone marrow morphology.**

Results. **Histopathology revealed that our cohort of patients could be divided into three distinct groups (true ET, questionable ET and false ET). These groups were characterized by certain diagnostic constellations of clinical data on admission. True ET was found in 53 patients presenting with no or a borderline splenomegaly and no relevant anemia or leuko-erythroblastic blood picture. The other patients showed clinical signs and symptoms which were more compatible with initial-prefibrotic (52 patients) or early (68 patients) idiopathic-primary myelofibrosis (IMF) with severe thrombocythemia. In true ET no significant hypercellularity of the bone marrow including myeloid precursors or an increase in reticulin fibers was detectable. Most prominent were changes of megakaryopoiesis which revealed large to giant-sized cells lacking a definite maturation defect. Their appearance in true ET contrasted with the clusters of abnormally differentiated, often bizarre elements of this lineage in patients with initial and early IMF (questionable or false ET). Calculation of survival disclosed a relevant disparity with a non-significant loss in life expectancy of 10.9% in true ET compared to 29.6% in questionable and 51.3% in false ET. Follow-up studies and repeated bone marrow biopsies revealed no transition into myelofibrosis in true ET, whereas this did occur in 22 of 27 patients with questionable and false ET. In the latter cohort bone marrow changes were accompanied by increasing anemia, splenomegaly, tear-drop poikilocytosis and reduction of the platelet count consistent with IMF.**

Interpretation and Conclusions. **A detailed evaluation of bone marrow features, in particular megakaryopoiesis is recommended to establish positive criteria for the diagnosis of ET and thus to accomplish a significant improvement of the PVSG postulates. In this context ongoing clinical trials on ET must regard pretreatment bone marrow biopsies as a major clue to diagnosis.**
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Key words: essential thrombocythemia, early myelofibrosis, diagnostic criteria, megakaryopoiesis, survival, bone marrow biopsies

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Essential (primary, hemorrhagic) thrombocythemia (ET) is a relatively rare chronic myeloproliferative disorder (MPD) characterized by a sustained elevation of the platelet count and an increased incidence of thromboembolic or hemorrhagic episodes.[1-5] Although most patients are asymptomatic at diagnosis[3,5] and the overall survival is comparable to that of the general population,[5-7] controversy and discussion still persist regarding stringent diagnostic criteria. There have been many years of arguments about realiable guidelines to establish ET as well as great difficulties in designing randomized therapeutic trials.[4,8] In the context of relatively ill-defined parameters within the spectrum of MPDs, ET remains a diagnosis of exclusion.[1,2,5,8,9] This fact has been appreciated by the *Polycythemia Vera Study Group* (PVSG) and finally emphasized by their updated criteria.[10] On the other hand, regarding these widely accepted postulates which have been generally used in clinical studies,[11-21] some authors have recently called attention to an obvious shortcoming which may impair diagnostic accuracy. This point includes a more scrutinized definition of morphologic parameters and thus attempts have been made to revise the original criteria by

the PVSG.[22] In keeping with this approach, a detailed analysis of histologic features in a series of patients with ET according to the PVSG criteria[10] resulted in a separation of two groups showing different bone marrow morphology (reticulin fibrosis, myeloid precursors, abnormalities of megakaryopoiesis) and overall survival.[23] Bearing these caveats in mind it still remains questionable whether histomorphologic features have a discriminating effect[2,5,9,23–29] or whether the updated criteria of the PVSG[10] are stringent enough to facilitate a clear-cut distinction of ET from the other subtypes of MPDs accompanied by thrombocythemia. The purpose of this retrospective study on 168 patients presenting with ET according to the updated PVSG criteria[4,5,9,10] was to analyze bone marrow morphology more systematically including follow-up examinations and associated clinical data. We further attempted an improvement of positive diagnostic criteria, in particular a clear-cut separation from early stage idiopathic myelofibrosis (IMF) in some cases accompanied by severe thrombocythemia.

Design and Methods

This retrospective clinicopathologic study was based on 168 adult patients selected from 498 cases with MPD and accompanying thrombocythemia who presented between 1982 and 1994 with findings in strict accordance with the updated criteria of the PVSG for the diagnosis of ET.[4,8-10] These included: a platelet count ≥ 600x10^9/L determined at intervals of at least two months, a hematocrit < 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 volume and finally, no collagen fibrosis or <1/3 biopsy area without both marked splenomegaly and leuko-erythroblastic reaction (Table 1). Patients entered the hospital or clinics with the following historical findings or complaints: episodes of bleedings (12%) ranging from easy bruising to major hemorrhage, arterial and deep venous thrombosis (10%), non-specific symptoms such as fatigue, weight loss, frequent infections and functional vasomotor symptoms (21%), abdominal distress (7%), night sweats (5%) and bone pain (4%). At least one representative bone marrow trephine biopsy was performed at diagnosis and in 44 patients a second examination at a mean interval of 45 months (range 6-131 months) was carried out. Therapy was based on age, presence of a poor performance status and complications such as hemorrhage and thromboembolic episodes and was as follows: 122 asymptomatic patients had either no treatment (34 patients) or a continuous, occasionally also intermittent administration of antiplatelet drugs (inhibitors of platelet aggregation-aspirin and derivates) alone (64 patients) or in combination with the other regimens (24 patients). The remaining 46 patients received single agent oral chemotherapy including hydroxyurea (19 patients), busulfan (14 patients), cytosine arabinoside and interferon-α2b (2 patients) or various combinations of these or other drugs (21 patients).

Table 1. Clinical findings in 168 patients with ET according to the updated diagnostic criteria of the PVSG.[4,5,8-10]

	Mean ± SD	*Range*
Gender (male/female)	74/94	-
Age (median; years)	62	24 -86
Thrombocytes (x 10^9/L)	1,118±830	600-10,240
Erythroblasts (%)	0.07±0.02	0-2
Myeloblasts (%)	0.06±0.04	0-4
Spleen size*	1.1±1.6	0-8
LAP°	81±78	0-329
LDH (U/L)	288±92	91-726
Erythrocytes (x 10^{12}/L)	4.5±0.8	2.2-6.8
Hemoglobin (g/dL)		
Males	13.9±2.1	7.0-18.4
Females	12.7±2.0	7.0-17.2
Hematocrit (%)		
Males	40.7±6.0	24-50
Females	38.9±5.6	25-47
Leukocytes (x 10^9/L)	13.0±8.6	4.2-44.3
Survival (months)	139	-
Relative survival		
5 years	0.91	–
10 years	0.81	–
Loss of life expectancy (%)	13.5	-

**cm below costal margin; °leukocyte alkaline phosphatase- normal score 10-80*

Bone marrow biopsies

Representative trephine biopsies of the bone marrow (length 15.2 ± 1.9 mm) were taken from the posterior iliac crest at diagnosis or at sequential examinations. 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 ethylendiamine tetra-acetic acid (EDTA), pH 7.2, paraffin embedding, and employment of several routine staining techniques, involving Giemsa, PAS (periodic acid Schiff reagent), naphthol-AS-D-chloroacetate esterase, Perls' reaction for iron and the silver impregnation method, following Gomori's technique. For a more specific staining of the different hematopoietic cell lineages in a number of patients two monoclonal antibodies

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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 mark erythropoiesis.[30] Monoclonal antibodies and other reagents were purchased from Dako-Diagnostica GmbH (Hamburg, Germany). Details of staining procedures (APAAP-method) were reported in previous communications.[28,31] As a control group we included a further 40 patients (22 males, 18 females; age 57 years) with reactive thrombocytosis (platelet count exceeding 480x10^9/L) due to diverse inflammatory conditions, splenectomy, iron-deficiency anemia and various metastasizing malignancies.

Statistical analysis

Evaluation included calculation of the non-parametric Mann-Whitney U-test to assess significant differences in the expression of clinical and hematologic variables on admission among the defined three groups of patients. Relative survival rates were determined to eliminate the effect of mortality from other, age-related causes than the underlying disorders. This calculation describes the ratio of observed to expected survival rates among individuals in the general population, corresponding to the patient group with regard to age, gender and calendar period of observation.[32,33] Calculations of the expected survival rates were obtained from life tables of the National Mortality Statistics (Germany). Moreover, to measure the impact of disease, life expectancies and the proportion of life loss were determined.[32,34]

Classification into subgroups

Evaluation of slides was carried out by three people in an independent fashion without prior knowledge of specific clinical data, follow-up examinations or survival. Biopsy specimens were referred with the presumptive diagnosis of ET established according to the well-known PVSG criteria.[4,5,8-10] However, following scrutinized morphologic analysis it turned out that the total cohort of 168 patients with ET diagnosed according to these postulates (Table 1) obviously constituted a heterogeneous population. In keeping with findings reported in the current literature on bone marrow histopathology in the different subtypes of MPDs, especially prefibrotic and early stage IMF,[24,25,28,29] recognition of distinctive patterns was possible. Therefore, as outlined in more detail in Table 2 designation to a certain entity could be accomplished. Concerning classification into the three groups minor disagreement among the hematopathologists regarded predominantly the exact amount of fibers (initial-early IMF) and not significantly the assessment of megakaryocyte atypias. Therefore a consensus was easily reached before clinical data were specified for the different categories under consideration.

Table 2. Survey of bone marrow features, in particular megakaryocyte morphology between true ET and initial-early IMF. ***Semiquantitative grading - N: normal amount, -: slight reduction, +: slight increase (up to two-fold of the normal), ++: moderate increase, +++: significant increase.***

Bone marrow features	*True ET* group I	*Questionable ET (prefibrotic IMF)* group II	*False ET (early fibrotic IMF)* group III
Cellularity	N	+	+
Neutrophil granulopoiesis	N	+	+
Erythropoiesis	N	-	-
Fibers (reticulin)	N	N	+
Megakaryopoiesis			
- quantity	+++	++/+++	++/+++
- prominent clusters	-	+	+
size	large to giant	median to giant	
nucleus	staghorn-like, deep lobulation	reduced and clumsy lobulation, cloud-like features, hyperchromasia	
dysplasia (maturation defect)	no relevant nuclear-cytoplasmic aberration	marked nuclear-cytoplasmic deviation	

Results

In keeping with predominant bone marrow features which are described in Table 2 separation of our total cohort of 168 patients into different groups (I-III) was feasible. Patients assigned to the category of true ET showed no relevant hypercellularity in comparison to cellularity of an age-matched population and also failed to exhibit immaturity of the granulocytic or erythrocytic lineages (Figure 1a). On the other hand, so-called questionable and false ET (groups II and III, respectively) were characterized by a prominent neutrophil granulocytic proliferation including myeloid precursors (Figure 1b). In comparison with true ET (Figure 2a) there was a slight reduction of the left-shifted and occasionally macrocytic erythropoiesis in false ET compatible with early IMF (Figure 2b). No increase in reticulin fibers was recognizable in true and in questionable ET corresponding with prefibrotic IMF (Figure 3a). Contrasting this finding in the third group of patients (false ET) a borderline to slight reticulin fibrosis, but no collagen could be found (Figure 3b). However, in comparison with reactive thrombocytosis (control group) the most conspicuous differences were those concerning megakaryopoiesis (Table 2). In patients of our control group a moderate

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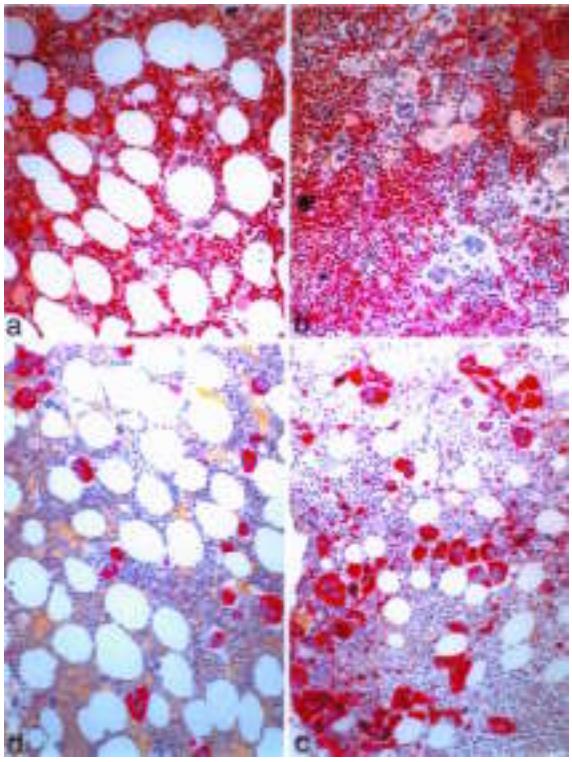
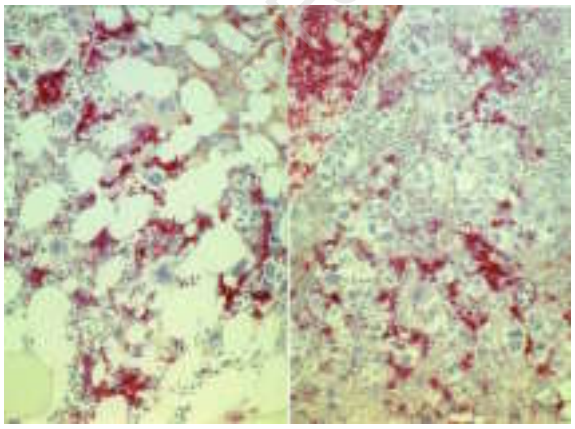
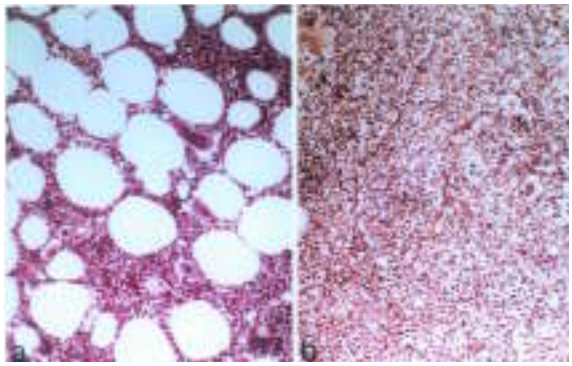


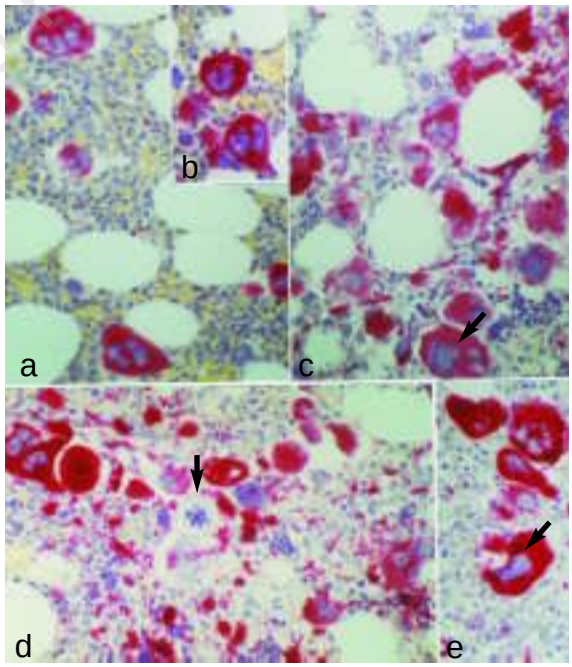
Figures 1a-d. Group I (true ET) patients have no conspicuous increase in cellularity and a normal ratio of granulo-to erythropoiesis (a) in contrast to group II (questionable ET) with mixed granulocytic and megakaryocytic growth (b). True ET is characterized by scattered large- to giant-sized megakaryocytes (c), whereas false ET (i.e. initial - early IMF with thrombocythemia) shows large clusters of abnormal (dysplastic) megakaryocytes (d). a and b: naphthol AS-D-chloroacetate stain, c and d: CD61 immunostaining, a-d x 170.



Figures 2a,b. Erythropoiesis shows inconspicuous small-to medium-sized groupings in true ET (a) in comparison to a slight reduction and occasionally a left-shifted and macrocytic appearance (inset) in initial IMF (false ET) with associated thrombocythemia and prominent megakaryocytic growth (b). a and b: Ret40f immunostaining, a and b x170.



Figures 3a,b. At onset group II patients with questionable ET consistent with prefibrotic IMF show no increase in reticulin (a), contrasting with an initial (reticulin) myelofibrosis which is focused around the vessels in false ET - early stage IMF (b). a and b: silver impregnation, a and b x170.



Figures 4a-e. In group I patients with true ET megakaryopoiesis is characterized by a prevalence of large to giant cells (a) containing extensively lobulated (staghorn-like) nuclei (b). In contrast to these features there is a variety of sizes in questionable ET consistent with prefibrotic IMF accompanied by thrombocythemia (c). Occurrence of bizarre forms with definite disturbances of maturation in false ET or initial-early IMF is prominent (d-e), often revealing polyploid endomitotic figures - arrow (d) or large clumsy or cloud-like (arrows) nuclei (c,e). a-e: CD61 immunostaining, a-e x570.

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to significant hyperplasia of small to medium-sized megakaryocytes was detectable, without, however, there being clustering or obvious cytological anomalies. On the other hand, true ET (group I) showed a prevalence of more or less diffusely scattered giant to large megakaryocytes with extensively lobulated staghorn-like nuclei surrounded by a correspondingly developed portion of well-differentiated cytoplasm (Figures 1c, 4a,b). Compared to these changes, cases of questionable or false ET (groups II, III - prefibrotic and early IMF) revealed not only prominent clustering, but also pronounced abnormalities of megakaryocyte differentiation (Figures 1d,4b). The latter resulted in a deviation of nuclear-cytoplasmic maturation (dysplastic changes) and thus frequently generated bizarre cells (Figures 4d,e). Most conspicuous was a clumsy appearance of nuclear lobulation often associated with hyperchromasia and a cloud-like aspect (Figure 4e). Altogether, megakaryocytic atypias were the most remarkedly expressed alterations to distinguish true ET from prefibrotic or early IMF (Table 2).

Clinical findings of the patients at diagnosis are shown in Table 3 for the three histological subgroups (Table 2) initially assumed to have presented with ET. Constellations of laboratory data (Table 4) and follow-up examinations suggested that group II and III patients had diagnoses were consistent with initial to early IMF and associated thrombocythemia. Observed and relative survival rates were different in these groups; in particular, expectation of life loss (Table 3) showed no significant reduction in those patients assumed to have true ET (group I). In this context sequential examinations in 14 patients of group II with so-called questionable ET revealed a transition into early to advanced fibro-osteosclerotic IMF in 13 cases within 60 months from diagnosis. Moreover, the categorization of having presented with false ET, assigned to 13 patients, was supported by the finding of progressively developing myelofibrosis in 9 of them during the observation time (28 months). These changes in bone marrow morphology were accompanied by laboratory parameters consistent with manifest IMF (i.e. teardrop poikilocytosis, anemia, splenomegaly, reduction in the platelet count). Contrasting this course, in the 17 patients of group I with true ET, in whom repeated bone marrow biopsies (mean interval 25 months) could be performed, no such alterations were detectable.

Discussion

The findings presented in this study are in keeping with the assumption that the updated and allied criteria of the PVSG for diagnosis of ET[4,5,8-10] are not stringent enough to exclude initial-early cases of IMF accompanied by thrombocythemia. This fact is not surprising, since a more critical evaluation of the original[1,2,9] and even the revisited[10] postulates reveals that the discriminating impact is focused on CML and PV, but not explicitly on IMF. Patients with early stage IMF

Table 3. Revised clinical data of the different groups of 168 patients presenting with a presumed diagnosis of ET following histomorphologic evaluation.

Final diagnosis	*True ET group I*	*Questionable ET (prefibrotic IMF) group II*	*False ET (early fibrotic IMF) group III*
Number of patients	53	52	63
Gender (male/female)	17/36	27/25	30/33
Age (median; years)	55	65	66
Thrombocytes (x 10^9/L)	1,271±513	1,021±438	1,072±1,202
Erythroblasts (%)	0.06±0.03	0.07±0.03	0.08±0.04
Myeloblasts (%)	0	0	0.1±0.6
Spleen size*	0.5±1.4	1.0±1.5	1.5±1.7
LAP°	59±44	92±82	124±86
LDH (U/L)	297 ±101	270±67	296±103
Erythrocytes (x10^{12}/L)	4.6±0.7	4.6±0.8	4.5±0.9
Hemoglobin (g/dL)			
Males	15.0±2.1	13.9±1.7	13.5±2.4
Females	13.1±1.7	12.5±2.4	12.7±2.1
Hematocrit (%)			
Males	43.5±6.3	42.5±4.6	41.7±6.9
Females	40.5±5.7	39.9±5.5	37.9±6.4
Leukocytes (x 10^9/L)	11.7±4.6	14.2±13.3	12.9±10.3
Survival (months)	170	133	105
Relative survival			
5 years	1.00	0.82	0.68
10 years	0.98	0.62	0.63
Loss of life expectancy (%)	10.9	29.6	51.3

**cm below costal margin; °leukocyte alkaline phosphatase; normal score 10-80.*

Table 4. Constellation of some major laboratory data (%) in the different groups of patients with ET according to the diagnostic criteria of the PVSG.[4,5,8,10] * *Significant ($p < 0.05$) difference in comparison to true ET (group I).*

Final diagnosis	*True ET group I*	*Questionable ET (prefibrotic IMF) group II*	*False ET (early fibrotic IMF) group III*
Thrombocytes ≥ 1,000 x 10^9/L	61	41*	40*
Splenomegaly ≥ 2 mm below costal margin	8	15*	35*
LAPL 80 (score)	17	23*	48*
Anemia Hemoglobin g/dL ♂ ≤ 14.0; ♀ ≤ 12.5	30	40	45*
LDH ≥ 300 U/L	19	21	33*

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Table 5. Modified criteria for the diagnosis of (true) ET.

I. Positive criteria
1. Platelet count: sustained elevation (≥ 600 x10^9/L)
2. Histopathology: increased numbers of enlarged, mature megakaryocytes showing deeply-lobulated nuclei, no cytological abnormalities or promi nent clustering. No marked proliferation or immaturity of granulopoiesis or erythroid precursors. No or borderline reticulin and no collagen fibro sis.

II. Criteria of exclusion - no evidence of
3. Polycythemia vera
4. Chronic myeloid leukemia
5. Idiopathic myelofibrosis, early stage
6. Myelodysplastic syndromes (5q-)
7. Reactive thrombocytosis

were not definitively distinguished at onset, because of the inclusion of some degree of (collagen) fibrosis or splenomegaly and a leuko-erythroblastic reaction.[10] In this context, megakaryocyte morphology which was repeatedly recorded as one of the most important features of distinctive value was apparently neglected.[23-29,35,36] For this reason, it is understandable that of the 91 patients selected by the PVSG,[10] who originally entered the therapeutic trial on ET with a platelet count greater than 1,000 x 10^9/L, only 50 cases fulfilled all criteria for this disorder (true ET category). Moreover, in this context it has been recognized that the four patients who presented with mild marrow fibrosis, splenomegaly and leukoerythroblastosis appeared to constitute a histologically different subgroup at high risk of development of acute leukemia.[10] This impression of a heterogeneity of ET patients diagnosed according to the criteria of the PVGS[1-5,8-10] was further supported by a recently published study on bone marrow findings and their prognostic impact in 93 patients from the Italian ET Study Group.[23] In this study which was confirmed and extended by the present investigation cluster analysis of relevant parameters resulted in distinct histologic patterns associated with significantly different survivals of the patients. Evaluation of bone marrow features revealed that 40 patients could be distinguished who showed an increased cellularity including all cell lineages and myeloid precursors, a high content of reticulin fibers and trapped *dysplastic* megakaryocytes.[23] These results fit well with our assumption that a considerable number of patients assumed to present with ET should be diagnosed as having initial-early IMF complicated by evolving myelofibrosis and thus have an unfavorable prognosis (Table 3). Regarding separation from reactive thrombocytosis, clonality studies could present new positive criteria for ET diagnosis, at least in female patients.[37,38]

According to anecdotal experience and small series of patients, transition of ET into blastic crisis was reported in ranges varying between 2-11%.[10-13,39,40] In addition, marked increase in reticulin has been observed in a small number of ET cases preceding transformation into acute leukemia.[40] Contrasting these data, which are suggested to mirror an ill-defined separation between initial-hypercellular IMF and ET, a minimal to slight reticulin fibrosis was found to occur in clear-cut ET at a frequency of only 1-3% at onset.[29,35,41] This incidence is derived from a large referral center, where up to now trephine biopsies of 575 patients with ET have been evaluated.[29] In follow-up studies relatively few changes of the histologic pattern could be ascertained and an evolution into (reticulin) myelofibrosis was reported in about 5 to 9% of the patients within 5.5 years of observation,[35,41] and a blastic crisis in only 2 of 60 patients.[35] These data which are strikingly opposed to some pertinent reports in the current literature on ET[10-13] were explicitly based on a scrutinized evaluation of bone marrow morphology. On the other hand, early hypercellular IMF accompanied by thrombocythemia is characterized by a significant tendency to progress into collagen myelofibrosis and blastic crisis.[26,41-43] In manifest IMF an observed survival ranging between three to five years has been recorded in larger series of patients investigated.[44-46] Prognosis of initial prefibrotic and early hypercellular IMF, corresponding to our group of false ET, was recorded to be more favorable.[34]

As has already been mentioned, a number of authors have drawn attention to the fact that the salient point to improve the diagnostic criteria of the PVSG for ET is the recognition of megakaryocyte morphology.[22-26,28,29,35,36] The latter is believed to be significantly different from IMF and thus provides a diagnostic hallmark to recognize true ET.[28,35,36,47,48] Additionally, the arbitrarily chosen platelet limit of 1,000 x10^9/L[1] or 600x10^9/L[10] should be revised, because early-initial stages of ET may be overlooked. Recently concerns have been raised about whether these cut-off points can be used as reliable diagnostic criteria for ET and whether diagnosis might be made in patients with a thrombocyte count > 400x10^9/L.[49,50] The major problem in clinical practice with this approach is that these platelet counts are frequently encountered and that patients at the extreme end of the physiologic ranges or even with reactive thrombocytosis will undergo unnecessary and sometimes harmful investigations. In the evolution of the disease process there is an initial period when the platelet count still falls within the reference range; however, very occasionally patients may develop vascular complications and only later is the diagnosis of ET established when throm-

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bocytes rise above this limit.[49] When considering megakaryocyte morphology as one of the major diagnostic features, recognition of specific features characterizing early stages in the various subtypes of MPDs is warranted.[24,25,28,35,48] This regards especially differentiation from PV[9,46] and initial-prefibrotic IMF.[28,29,41,47,48] On the other hand, descriptions of megakaryopoiesis in the trials conducted by the PVSG were rather limited and focused on the term *hyperplasia*, but hardly recorded details of histopathology.[1,2,5,10] More detailed evaluations of cytological features of discriminating impact have repeatedly shown that, compared to normal appearing megakaryocytes in reactive, frequently occurring thrombocytosis,[24,25] in PV this cell lineage is characterized by a pleiomorphic appearance. Here clusters of giant megakaryocytes with extensively lobulated nuclei are mingled with medium-sized and small ones.[27,28,35,36,48] Contrasting this peculiar aspect of megakaryo-cytes in PV, initial-early IMF with associated thrombocythemic cell counts reveal an aberration of nuclear-cytoplasmic differentiation and maturation generally compatible with a dysplasia.[28,29,36,41,42,47,48] Compatible changes have been recently recognized by Annaloro and his co-workers when analyzing presumed ET cases diagnosed in accordance with the PVSG criteria.[23] In (true) ET megakaryocytes are mostly giant to large-sized and contain a correspondingly enlarged nucleus with deep (staghorn-like) lobulations, but lack the cytological abnormalities generally encountered in IMF.[35,36,41,42,47,48] Therefore in a significant number of cases initially regarded as ET the distinctive appearance of the megakaryocytic lineage and other bone marrow features (Table 2) should move the diagnosis to early hypercellular IMF as was accomplished in this study.

In addition to specific megakaryocyte morphology characterizing MPDs with accompanying thrombocythemia,[36] PV exhibits an increased cellularity or panmyelosis consistent with a trilineage proliferation of erythro- granulo- and megakaryopoiesis.[24-27,29,48] In initial-early IMF a neutrophil granulocytic plus megakaryocytic proliferation characterizes the most outstanding bone marrow changes and for this reason, has been coined chronic megakaryocytic-granulocytic myelosis.[25,28,29,48] Concerning involvement of all the other lineages, patients with ET show no significant increase or immaturity of neutrophil granulo- or erythropoiesis and have a normal hemosiderin content in their macrophages.[24,25,28,29,35,36,48] Taking these considerations into account, diagnosis of ET should not be based on the exclusion of the other subtypes of MPDs as has been principally done by the PSVG,[4,5,8-10] but must regard positive criteria i.e. bone marrow morphology as a most prominent feature of distinction (Table 5).

In this context the arbitrarily chosen limit of the platelet value ($\geq 600\times10^9$/L) is open to discussion, in particular in patients showing thrombotic or hemorrhagic complications.[40,50]

In conclusion, a more refined evaluation of certain bone marrow features, especially megakaryocyte morphology, is warranted to improve the diagnostic impact of the PVSG criteria for ET, in order to exclude initial to early (hypercellular) stages of IMF with a significantly unfavorable prognosis. Keeping this in mind, ongoing clinical trials must regard representative (pretreatment) bone marrow biopsies as a major clue to the diagnosis of ET. Moreover, retro- and prospective double-blind studies should be initiated to discriminate groups of patients according to the histologic features described in our cohort of patients originally having been assumed to have presented with ET.

Contributions and Acknowledgments

JT designed the study, reviewed the bone marrow biopsies, contributed to the interpretation of data and drafted the article. HMK reviewed the bone marrow biopsies, revised the article and performed the statistical analysis and contributed to the interpretation of the data. RZ and VD were involved in the collection and interpretation of clinical-laboratory data including follow-up examinations of patients. The authors are listed according to the importance of their contribution to the work. The last name is that of the principal clinician involved.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

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Potential implications for clinical practice

- Ongoing clinical trials on ET must regard representative (pretreatment) bone marrow biopsies as a major criterion for entry into any study.
- Series of patients with ET revealing borderline to moderate anemia, splenomegaly or transition into myelofibrosis on follow-up examinations should be revised more critically, i.e. the initial bone marrow biopsy should be reviewed to exclude prefibrotic IMF with thrombocythemia (questionable/false ET).
- A (retro- and prospective) double-blind study should be initiated (perhaps including the excessive number of patients from the Italian ET Study Group) to discriminate groups of patients according to their histomorphology (true, questionable, false ET) and corresponding sets of clinical-laboraory data including follow-ups.

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